PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 5/02, 5/10, 7/02, 7/08, 7/10, C07D 39/70, A61K 31/495, 37/02, 37/64, 39/00 — A1

(11) International Publication Number: WO 94/17095

(43) International Publication Date: 4 August 1994 (04.08.94)

(21) International Application Number: PCT/US94/01025

(22) International Filing Date: 28 January 1994 (28.01.94)

(30) Priority Data:
013,414 29 January 1993 (29.01.93) US

(71) Applicant: ARIAD PHARMACEUTICALS, INC. [US/US]; 26 Landsdowne Street, Cambridge, MA 02139 (US).

(72) Inventors: WEIGELE, Manfred; 6 Canal Park, Cambridge, MA 02141 (US). TAO, Guo; 41 Belmont Street No. 32, Somerville, MA 02143 (US). SUNDARAMOORTHI, Raji; 1 Bailey Place, 120 1/2 Magazine Street, Cambridge, MA 02139 (US). DALGARNO, David, C.; Apartment 15, 90 Commonwealth Avenue, Boston, MA 02116 (US). ZYDOWSKY, Lynne, D.; 10 Rogers Street #410, Cambridge, MA 02142 (US). GREEN, Jeremy; 1313 Arboretum Way, Burlington, MA 01803 (US). GREEN, Oluyinka, M.; 1313 Arboretum Way, Burlington, MA 01803 (US).

(74) Agents: MISROCK, S. Leslie et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KR, KZ, LK, LV, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ANALOGS OF RECEPTOR TYROSINE ACTIVATION MOTIFS AND THERAPEUTIC USES THEREOF

(57) Abstract

The present invention is directed to peptides representing all or a portion of tyrosine activation motifs (TAMs), as well as analogs and derivatives thereof (collectively termed herein "TAM Mimics"). In particular, such analogs and derivatives include peptides and hybrid molecules with both peptide and non-peptide portions. In a specific embodiment, a hybrid molecule is provided which contains an amino(N)- or carboxy(C)-terminal non-peptide helix inducer. In another specific embodiment, the hybrid molecule contains an internal non-peptide structure that maintains the helical character of the TAM mimic. TAM Mimics of the invention inhibit the activation of various cells with immune system function. Therapeutic methods based on the immune function inhibitory activity of the TAM Mimics, as well as pharmaceutical compositions, are also provided. In a preferred embodiment of the invention, TAM Mimics are formulated for pulmonary administration; thus, for example, the invention provides inhalers containing compositions comprising a therapeutically or prophylactically effective amount of a TAM Mimic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

ANALOGS OF RECEPTOR TYROSINE ACTIVATION MOTIFS AND THERAPEUTIC USES THEREOF

1. INTRODUCTION

The present invention relates to peptides and analogs and derivatives thereof that are related to all or a portion of tyrosine activation motifs found in multichain immune recognition receptors. Such peptides, analogs, and derivatives have utility in the treatment of diseases or disorders associated with undesirable or inappropriate immune activity or inflammation.

2. BACKGROUND OF THE INVENTION

2.1. IMMUNOGLOBINS AND THE IMMUNE RESPONSE

The immune system consists of lymphocytes, macrophages, and other specialized cells. An organism responds to antigen by virtue of having a large number of lymphocytes, each bearing receptors which recognize distinct antigens. B lymphocytes are precursors of antibody-secreting cells, and T lymphocytes are involved in regulatory and effector functions. In addition, natural killer cells mediate certain cytotoxic responses. (See generally, *Fundamental Immunology*, 1989, 2d Ed., Paul, W.E. (ed.), Raven Press Ltd., New York; Benacerraf & Unanue, 1979, *Textbook of Immunology*, Williams and Wilkins, Baltimore).

Cellular (or cell-mediated) immunity results from the activity of T lymphocytes that have been sensitized to an antigen by virtue of their recognition of the antigen via their T cell antigen receptor complex (see generally, Hedrick, 1989, in *Fundamental Immunology*, 2d Ed., ch. 11, Paul, W.E. (ed.), Raven Press Ltd., New York, pp. 291-313; Weiss,

1989, in *Fundamental Immunology*, 2d Ed., ch. 13, Paul, W.E. (ed.), Raven Press Ltd., New York, pp. 359-384).

Antibody or humoral immunity is mediated by molecules called antibodies that combine specifically with antigens; immunoglobulins are proteins with antibody activity. There are five classes of human immunoglobulins, differing in the structure of their respective heavy chains: IgG, IgA, IgM, IgD, and IgE. Immunoglobulins are synthesized by B lymphocytes, and by their progeny, the plasma cells. B cells interact with antigen via the antibody molecules in their plasma membranes. Plasma cells secrete antibodies. IgGs are the major component of serum immunoglobulin, and the predominant antibody involved in a secondary immune response. IgM is the predominant antibody involved in a primary immune response. IgD is a cell surface receptor present on mature B cells. IgA is found in bodily secretions. IgE is found in normal human serum at very low concentrations, and is involved in type I allergic reactions (atopy).

IgE appears to mediate local and systemic immediate hypersensitivity and anaphylactic reactions. Atopic individuals produce IgE in response to many environmental antigens. The event that initiates immediate hypersensitivity in such patients is the binding of antigen to IgE on the surface of mast cells. Binding of the Fc domain of the allergen-bound IgE to the extracellular domain of the FcεRI receptor complex on mast cells and basophils induces crosslinking of the receptor. Crosslinking of IgE receptors (FcεRI) causes mast cell activation and a cascade of intracellular signaling events which ultimately results in the release of lipid mediators of inflammation (leukotrienes, etc.), proinflammatory cytokines (interleukins, etc.) and of secretory

granules (containing histamine, 5-hydroxytryptamine, hexosaminidase, etc.). This release causes the appearance of many of the symptoms of acute allergic response.

2.2. MULTICHAIN IMMUNE RECOGNITION RECEPTORS

Cells respond to external stimuli through the binding of extracellular ligands to transmembrane receptor molecules. Binding of protein or peptide ligands to the extracellular domain of such a membrane-spanning receptor results in a conformational change or aggregation of the receptor, and a subsequent relay of the conformational signal induced by the external stimulus to the intracellular portion of the receptor. Intracellular signal transduction occurs via changes in the intermolecular associations of protein molecules arranged in a complex multi-step sequential pathway leading from the intracellular portion of the receptor to proteins involved directly in controlling gene expression, cytoskeletal architecture and cell division (Cantley et al., 1991, Cell 64:281-302). Recent research shows that signal transduction is accomplished at a molecular level via protein-protein complex formation, or, more specifically, by the creation of new protein-protein interaction sites, *e.g.*, by enzymatic modification of tyrosine residues or by conformational changes induced by binding. Specific examples of intracellular signaling events modulated by protein-protein interactions are receptor-coupled G-protein activation, immunosuppression via immunophilin-immunosuppressive agent complexes and the release of the contents of secretory vesicles, *e.g.*, histamine release in mast cell degranulation. Several protein-protein interaction sites involved in signaling have

been localized to short (less than 20 amino acid) contiguous sequences (see, *e.g.*, Koch et al., 1991, Science 252:668-674).

Multichain immune recognition receptors (MIRRs) (Keegan and Paul, 1992, Immunology Today 13:63-68) are found on T and B cells, mast cells, macrophages, natural killer cells and platelets. MIRRs constitute a family of immune cell receptors involved in the activation of cells involved in the immune system. MIRRs include the B cell antigen receptor complex (BCR); the T cell antigen receptor complex (TCR); FcγRIII (FcγRIIIA in humans), a receptor for the Fc region of IgG antibody molecules, found on mast cells, basophils, natural killer cells, macrophages, and neutrophils; and FcεRI, a receptor for the Fc region of IgE antibody molecules. These MIRRs consist of polypeptide chains that bind antigen or Fc, associated with chains whose intracellular domains contain a related short sequence of 18-27 amino acids termed the tyrosine activation motif ("TAM") (Samelson and Klausner, 1992, J. Biol. Chem. 267(35):24913-24916; Reth, 1989, Nature 338:383-384). The consensus sequence for the TAM is: (D/E)XXXXXX(D/E)XXYXXLXXXXXXXYXX(L/I)X (SEQ ID NO:1), where X is any amino acid (Reth, 1989, Nature 338:383-384). Figure 1 schematically depicts these MIRRs.

The TCR consists of the α (alpha) and β (beta) T cell antigen receptor antigen-binding chains (which, in a minority of T cells, are replaced by the γ (gamma) and δ (delta) heterodimer), the γ, δ and ε (epsilon) chains of the CD3 complex, and a disulfide linked ζ (zeta) dimer or heterodimer of ζ and η (eta) chains. The BCR consists of the heavy and light chains and at least one disulfide-linked

heterodimer consisting of Ig-α (MB-1 gene product) and Ig-β (B29 gene product). The FcεRI receptor is a c mplex of three proteins, the α, β, and γ subunits. The α subunit of FcεRI binds IgE, the β subunit contains four transmembrane regions, and the γ subunit consists of a part of disulphide-linked chains (Fig. 2).

Most of the FcεRI-γ subunit residues lie on the cytoplasmic side of the cell membrane. This γ subunit is shared by the FcγRIII MIRR, and contains a TAM motif. FcεRI-β also contains a TAM motif (but not FcεRI-α), as do the CD3-γ,CD3-δ and ζ components of the TCR (but not the CD3-ε or TCR-α or TCR-β), MB-1 and B29 in the BCR, and the envelope protein (gp30) of bovine leukemia virus (Reth, 1989, Nature 338:383-384; see also Fig. 3 herein).

The function of the TAM motif in signal transduction has been investigated for the TCR. The consensus sequence shown above occurs in the ζ chain of the TCR. Cell lines expressing a chimeric molecule in which the cytoplasmic tail of the ζ chain was attached to the unrelated CD8 receptor respond to antibody crosslinking stimuli (Romeo et al., 1992, Cell 68:889-897).

Citation of a reference herein shall not be construed as an admission that such reference is prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to peptides representing all or a portion of tyrosine activation motifs (TAMs), as well as analogs and derivatives thereof (collectively termed herein "TAM Mimics"). In particular, such analogs and derivatives include peptides and hybrid molecules with both

peptide and non-peptide portions. In a specific embodiment, a hybrid molecule is provided which contains an amino(N)- or carboxy(C)-t rminal non-peptide helix inducer. In another specific embodiment, the hybrid molecule contains an internal non-peptide structure that maintains the helical character of the TAM mimic. TAM Mimics of the invention inhibit the activation of various cells with immune system function.

Therapeutic methods based on the immune function inhibitory activity of the TAM Mimics, as well as pharmaceutical compositions, are also provided. In a preferred embodiment of the invention, TAM Mimics are formulated for pulmonary administration; thus, for example, the invention provides inhalers containing compositions comprising a therapeutically or prophylactically effective amount of a TAM Mimic.

4. DESCRIPTION OF THE FIGURES

Figure 1. Schematic diagram of four multichain immune recognition receptors (MIRRs): IgMR (the B cell antigen receptor complex), FcγRIII, EcεRI, and TCR (the T cell antigen receptor complex). Two FcεRI receptors are depicted cross-linked by allergen binding. Two IgMRs are depicted cross-linked by antigen binding. TAM motifs are indicated by cross-hatching.

Figure 2. A schematic view of the MIRR for the Fc region of IgE, called the FcεRI receptor (after Keegan and Paul, 1992, Immunology Today 13(2):63-68).

Figure 3. TAM Motifs in the cytoplasmic tail sequences of various MIRRs. h, human; r, rat; m, mouse.

Figure 4. Exemplary TAM Mimic compounds of Formula (V).

Figure 5. Computer-generated drawings. The left side shows an idealized (computer-generated) drawing of an alpha-helical peptide. In the structure on the right, 7 amino acids (*i.e.*, an 11 angstrom stretch) have been replaced by a "spacer molecule" like that present in a compound of formula (V).

Figure 6. Helix nucleating modules. N-terminal and C-terminal helix inducers are depicted. Compound I and Compound III were developed by Paul Bartlett (Bartlett et al., "Intuitive- and Computer-Assisted Approaches to the Design of Conformationally Restrained Peptides and Their Mimics," October 28-29, 1991, reprinted from Proceedings of The Robert A. Welch Foundation Conference on Chemical Research, XXXV Chemistry at the Frontiers of Medicine, Houston, Texas).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to peptides related to all or a portion of tyrosine activation motifs (TAMs), as well as analogs and derivatives thereof (collectively termed herein "TAM Mimics"). In particular, such analogs and derivatives include peptides and hybrid molecules with both peptide and non-peptide portions. In a specific embodiment, a hybrid molecule is provided which contains an amino(N)- or carboxy(C)-terminal non-peptide helix inducer. In another specific embodiment, the hybrid molecule contains an internal non-peptide structure that maintains the size and general shape of the TAM mimic. The TAM Mimics of the invention retain the conformational information of TAM motifs required for biological activity, however,

preferably incorporate sequence changes that provide the TAM Mimic with enhanced ability to cross lipid bilayers.

The TAM motif plays a critical role in the activation of the immune functions of T cells, B cells, mast cells, basophils, and natural killer (NK) cells. The TAM motif within receptor complexes appears to interact with target proteins that transmit the activation signal within the cytoplasm to bring about cellular immune responses. The TAM Mimics of the invention mimic the structure of these TAM motifs. While Applicants do not intend to be bound by any specific mechanism, Applicants believe that the TAM Mimics function by binding to the target protein, mimicking the interaction with the receptor-associated TAM motif, and thus preventing the natural activation of this target. Stimulation of mast cells, B lymphocytes, T lymphocytes, and macrophages is interfered with at the first stage in the stimulus-response coupling, thereby abrogating the responses of these cells.

The TAM Mimics of the invention thus act as inhibitors of the signal transduction events mediated by the various MIRRs containing TAM sequences, by competitively inhibiting the interaction of such molecules with an effector or regulatory molecule via their TAM motifs, and thus preventing the natural sequence of activation in MIRRs.

Therapeutic methods based on the immune function inhibitory activity of the TAM Mimics, as well as pharmaceutical compositions, are also provided. The TAM Mimics of the invention can also be used to study and elucidate the signal transduction mechanism in cells expressing the MIRRs.

5.1. TAM MIMICS

The TAM Mimics of the invention include peptides and hybrid molecules, as detailed more fully in the subsections below.

5.1.1. PEPTIDES

In a specific embodiment, the TAM Mimic is a peptide. Such a peptide can comprise all or a portion of a TAM motif actually present in a MIRR. For example, a TAM Mimic of the invention comprises or consists of all or a portion of the sequences shown in Figure 3 (SEQ ID NOS:2-12). In a preferred aspect, a TAM Mimic comprises or consists of the sequence (D/E)XXXXXX(D/E)XXYXXLXXXXXXXYXX(L/I)X (SEQ ID NO:1). In another embodiment, a TAM mimic comprises or consists of the following sequences:

(D/E)XXYXXLXXXXXXXYXX(L/I) (part of SEQ ID NO:1)
DGGYMTLNPRAPTDDDKNTYLTLP (part of SEQ ID NO:2)
DAVYTGLSTRNQETYETLK (part of SEQ ID NO:3)
DRLYEELHVYSPIYSALE (part of SEQ ID NO:4)
DQLYQPLKDREDDQYSHLQ (part of SEQ ID NO:5)
DQVYQPLRDRDDAQYSHLG (part of SEQ ID NO:6)
DGLYQGLSTATKDTDALH (part of SEQ ID NO:7, 8)
ENLYEGLNLDDCSMYEDIS (part of SEQ ID NO:9, 10)
DHTYEGLNIDQTATYEDIV (part of SEQ ID NO:11)
EHTYEGLNIDQTATYEDIV (part of SEQ ID NO:12)

In yet another specific embodiment, a TAM Mimic peptide comprises or consists of the β or γ TAM motif, or a portion thereof, present in the human FcϵRI complex, or one of the three TAM motifs, or a portion thereof, present in the human CD3 zeta chain; such TAM motifs are as follows:

FcϵRI β TAM motif:
E D R V Y E E L N I L S A T Y S E L E D P G E N
(SEQ ID NO:26)

FcεRI γ TAM motif:
S D G V Y T G L S T R N Q E T Y E T L K H E K
(SEQ ID NO:27)
CD3 ζ TAM motif #1:
Y Q Q G Q N Q L Y N E L N L G R R E E Y D V L D K R R
G R D P E M G G K P (SEQ ID NO:28)
CD3 ζ TAM motif #2:
R R K N P Q E G L Y N E L Q K D K M A E A Y S E I G M
K G E (SEQ ID NO:29)
CD3 ζ TAM motif #3:
R R R G K G H D G L Y Q G L S T A T K D T Y D A L H M
Q A L P P R (SEQ ID NO:30)

The TAM motifs from different receptor subunits appear to specifically activate distinct biological responses in different cell types. For example, the TAM motif from the ζ subunit of the T cell receptor is not expected to be as active in activating mast cells as in activating T cells, the γ subunit TAM motif from the IgE mast cell receptor is not expected to be as active in activating T cells as in activating mast cells. The amino acid in SEQ ID NO:1 other than X are invariant residues that are shared by all members of the TAM family. These shared residues must be important for a common function of all of these domains, whereas the variant residues provide a structural scaffold or contribute specificity to this domain (*i.e.*, recognition of different target proteins). Thus, TAM Mimics are also provided which specifically interfere with activation or immune responses in specific immune cells, by virtue of containing sequences of variant residues (*e.g.*, residue numbers 2-8, 10-11, 13-14, 16-22, and/or 24-25 in SEQ ID NO:1) which are identical or substantially the same as those sequences present in MIRRs (see Fig. 3).

In a preferred aspect, immune activation of mast cells and basophils via the allergen-IgE receptor complex is interfered with by use of TAM Mimics which mimic FcεRI γ or β TAM motifs. In a preferred aspect, a TAM mimic comprises one of the following sequences, related to the FcεRI γ TAM motifs:

D G V Y T G L S T R N Q E T Y E T L K (SEQ ID NO:19)

Q K V Y D K L L K R N Q E T Y E T L K (SEQ ID NO:14)

Q K V Y D K L L K R N Q E L Y E T L K (SEQ ID NO:15)

Q K V Y C K L L C R N Q E L Y E T L K (SEQ ID NO:16)

D G V Y T G L S T R N Q K T Y K T L K (SEQ ID NO:17)

N G V Y T G L S T R N Q K T Y K T L K (SEQ ID NO:18)

In the above-presented sequences, dashed lines represent potential salt bridges between the indicated residues; solid lines represent cyclization (covalent linkage) of the indicated residues. Cyclization in SEQ ID NO:15 is via an amide linkage between the indicated residues. Cyclization in SEQ ID NO:16 is via disulfide bond formation. The envisioned salt bridges and the cyclizations are preferably present in the indicated sequences because they are expected to increase the conformational stability of the peptide.

In a preferred aspect, the TAM Mimics consist of the following 19-mer peptides (Ac = acetyl; solid lines between residues indicates cyclization):

Ac-D G V Y T G L S T R N Q E T Y E T L K-NH_2 (SEQ ID NO:19);

Ac-Q K V Y D K L L K R N Q E T Y E T L K-NH_2 (SEQ ID NO:20);

Ac-Q K V Y D K L L K R N Q E L Y E T L K-NH_2 (SEQ ID NO:21);

Ac-Q K V Y C K L L C R N Q E L Y E T L K-NH_2 (SEQ ID NO:22);

Ac-D G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:23); and

Ac-N G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:24).

SEQ ID NO:13 is the actual sequence contained in the C-terminal region of human FcεRI-γ.

In another embodiment, a TAM Mimic comprises or consists of the sequence:

D X V Y X X L X X R N Q E X Y E T L K (SEQ ID NO:25)

TAM Mimics of the invention which have a sequence more homologous to a TAM motif found in an FcεRI γ or β chain than to other TAM motifs are preferred for inhibition of mast cell (or basophil cell) activation. TAM Mimics which have a sequence more homologous to a TAM motif found in FcγRIII (or FcγRIIIA) γ or ζ chain are preferred for inhibition of macrophage or natural killer cell activation. TAM Mimics which have a sequence more homologous to a TAM motif found in the B cell antigen receptor complex (MB-1 or B29) are preferred for inhibition of B cell activation. TAM Mimics which have a sequence more homologous to a TAM motif found in the CD3-γ, CD3-δ, or ζ chain of the T cell antigen receptor complex are preferred for inhibition of T cell activation.

The TAM Mimics of the invention can be derived from TAM motifs of proteins of human or other animal origin, including but not limited to mammals such as cows, horses, pigs, sheep, goats, rats, mice, dogs, chickens, rabbits, etc.

The peptide TAM Mimics of the invention have a sequence in the range of 15-39 amino acids. In a

specific aspect, the peptides have a sequence in the range of 17-25 amino acids. In a preferred aspect, the p ptides contain 19 amino acids.

The TAM mimic peptides of the invention preferably contain naturally-occurring amino acids. The most common naturally-occurring amino acids are shown in Table I:

TABLE I.

NATURAL AMINO ACIDS AND THEIR ABBREVIATIONS

Name	3-Letter Abbreviation	1-Letter Abbreviation
(+)-Alanine	Ala	A
(+)-Arginine	Arg	R
(-)-Asparagine	Asn	N
(+)-Aspartic acid	Asp	D
(-)-Cysteine	Cys	C
(+)-Glutamic acid	Glu	E
(+)-Glutamine	Gln	Q
Glycine	Gly	G
(-)-Histidine	His	H
(+)-Isoleucine	Ile	I
(-)-Leucine	Leu	L
(+)-Lysine	Lys	K
(-)-Methionine	Met	M
(-)-Phenylalanine	Phe	F
(-)-Proline	Pro	P
(-)-Serine	Ser	S
(-)-Threonine	Thr	T
(-)-Tryptophan	Trp	W
(-)-Tyrosine	Tyr	Y
(-)-Valine	Val	V

However, the peptides of the invention are not limited to containing the 20 natural amino acids. In other embodiments, TAM mimic peptides can contain non-natural amino acids or cyclic peptides. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be the D (dextrorotary) or L (levorotary) amino acid.

The peptide may be prepared by methods that are known in the art. For example, in brief, solid phase peptide synthesis consists of coupling the carboxyl group of the C-terminal amino acid to a resin and successively adding N-alpha protected amino acids. The protecting groups may be any known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The coupling of amino acids to appropriate resins is described by Rivier et al., U.S. Patent No. 4,244,946. Such solid phase syntheses have been described, for example, by Merrifield, 1964, J. Am. Chem. Soc. 85:2149; Vale et al., 1981, Science 213:1394-1397; Marki et al., 1981, J. Am. Chem. Soc. 103:3178 and in U.S. Patent Nos. 4,305,872 and 4,316,891. In a preferred aspect, an automated peptide synthesizer is employed.

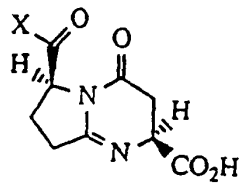
Purification of the synthesized peptides can be carried out by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation,

differential solubility, or by any other standard technique for the purification of proteins. In a preferred embodiment, reverse phase HPLC (high performance liquid chromatography) is employed.

5.2. HYBRID MOLECULES WITH AMINO- OR CARBOXY-TERMINAL HELIX INDUCERS

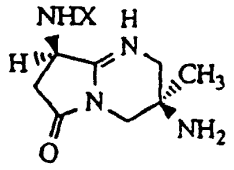
TAM Mimics of the invention also include hybrid molecules that comprise peptides bound to amino- or carboxy-terminal helix inducers. In particular, such hybrid molecules are TAM Mimic peptides, in which two amino acid residues at the N-terminus and/or C-terminus of the peptide are replaced respectively by an N-terminal or C-terminal helix inducer, or a helix inducer of 0-3 residues can be added. Such helix inducers are nonpeptide organic structures that function as helix nucleating modules. Examples of N-terminal and C-terminal helix inducers, respectively in C-terminal or N-terminal linkage to a peptide amino or carboxyl group respectively, are shown in Figure 6.

In a specific embodiment, the invention thus provides a compound of formula (IIc), wherein X is OH or an amino-terminally linked peptide having a sequence in the range of 17-39 amino acids and comprising the sequence V Y T G L S T R N Q E T Y E T L K (part of SEQ ID NO:13).



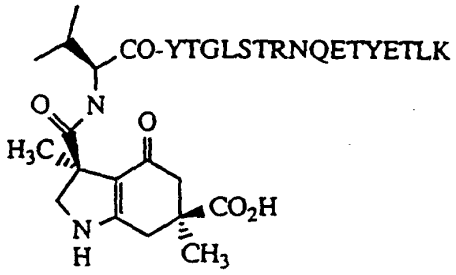
(IIc)

In another specific embodiment, the invention provides a compound of formula (IVa), wherein X is a hydrogen atom or a carboxy-terminally linked peptide having a sequence in the rang of 17-39 amino acids and comprising the sequence V Y T G L S T R N Q E T Y E T L K (part of SEQ ID NO:13).

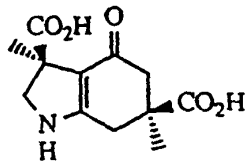


(IVa)

In a preferred aspect of the invention, hybrid peptide (Ia) is produced, containing helix inducer (Ib) replacing the N-terminal DG in the sequence of the TAM Mimic Peptide TAM-1 (see Section 6) having the sequence:
D G V Y T G L S T R N Q E T Y E T L K (SEQ ID NO:13)



(Ia)



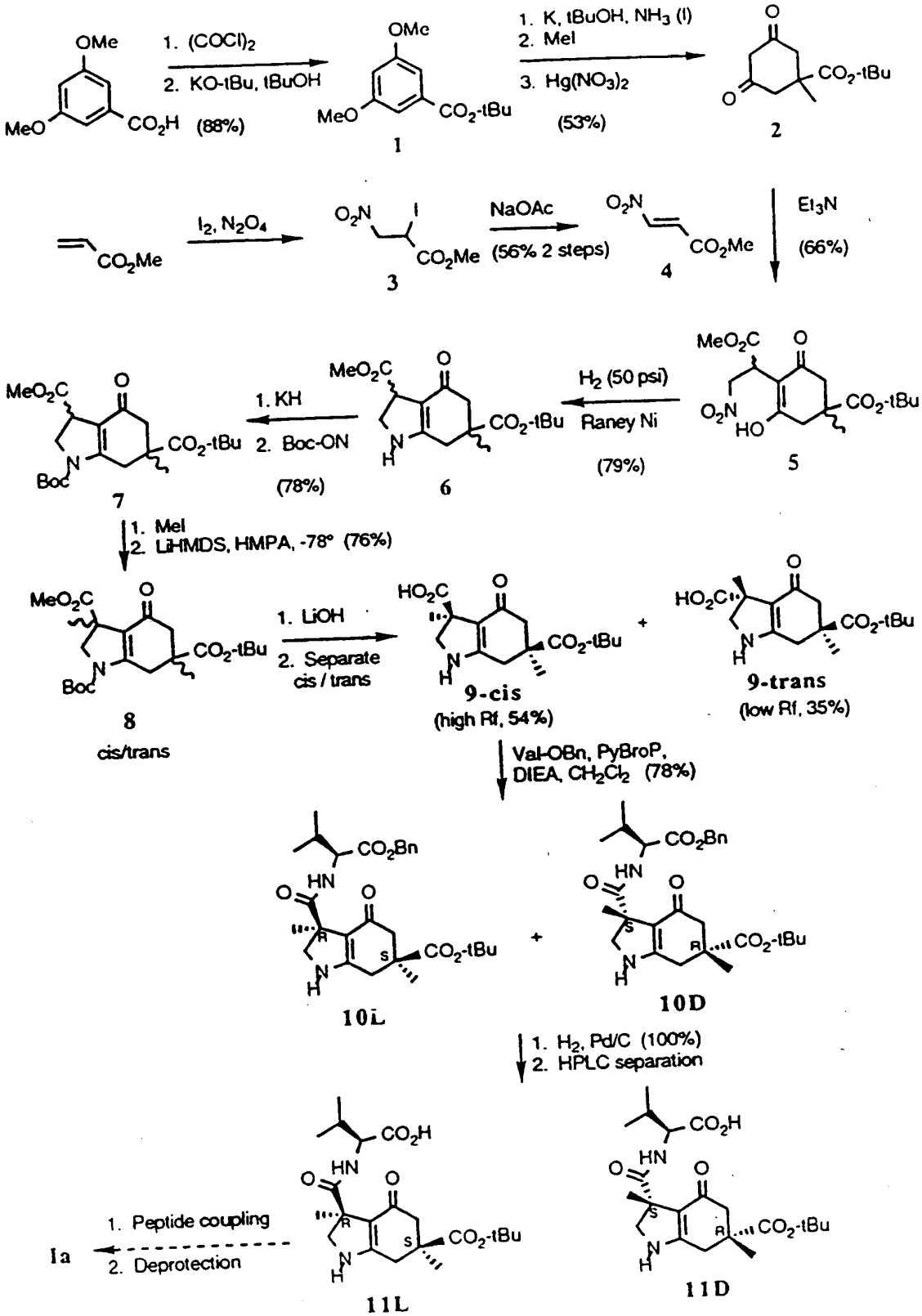
(Ib)

In specific embodiments, the C-terminus of compound (Ia) is the free acid, an ester (OR, where R is preferably an alkyl of 1-4 carbon atoms), or an amide.

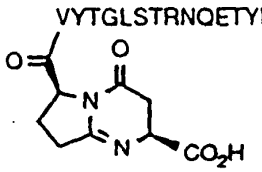
By way of example, hybrid peptide (Ia) may be prepared according to Scheme I. 3,5-Dimethoxybenzoic acid is converted to its acid chloride by treatment with oxalyl chloride in dichloromethane at 0°C to room temperature (r.t.) for 5 h. The acid chloride is esterified using potassium tert-butoxide in tert-butanol at 30-40°C for 22 h yielding the tert-butyl ester (1). Birch reductive alkylation of (1) with potassium in a mixture of tert-butanol and ammonia with iodomethane as the alkylating reagent at 78°C for 2 h followed by treatment with mercurous nitrate at reflux in aqueous acetonitrile for 14 h affords the di-keto ester (2). Treatment of (2) with nitro ester (4) and triethylamine in methanol at 0°C to r.t. for 2 d gives the hydroxy di-ester (5). Olefin (4) is synthesized from methyl acrylate by reaction with iodine and nitrogen dioxide at 0°C for 1 h and then at r.t. for 4 h yielding (3) followed by elimination of hydrogen iodide with sodium acetate in ether at reflux for 3 h to give (4). Reduction of the nitro group of (5) with hydrogen at 50 psi using Raney nickel in MeOH/EtOH affords the diester (6) via concomitant ring closure of the amine produced by the reduction of the nitro group. Deprotonation of the amine (6) with potassium hydride followed by protection with 2-tert-Butoxycarbonyl)-2-tert-butyl-3-methyl-4-imidazolidinone yields the protected amine (7). Treatment of (7) with lithium hexamethyldisilazane and hexamethylphosphoramide in tetrahydrofuran at -78°C leads to the formation of the methyl ester enolate which is subsequently alkylated with iodomethane to yield a mixture of cis/trans (8). Selective hydrolysis of the methyl ester of (8) using lithium hydroxide followed by silica gel chromatography separation gives the desired cis-

diastereomer (9). The cis-acid (9) is coupled to L-valine benzyl ester using bromo-tris-pyrrolidino-phosphonium hexafluorophosphate and diisopropyl ethylamine in dichloromethane at 0°C to r.t. for 16 h to give a 1:1 mixture of diastereomers 10L and 10D. Hydrogenolysis of the mixture using hydrogen and a catalytic amount Pd/C followed by HPLC on a reverse phase column provides the desired diastereamer 11L along with diastereomer 11D. Coupling of 11L to TAM peptide 16-mer followed by removal of side chain protecting groups gives the desired hybrid peptide (Ia). The above-described synthetic scheme has been carried out as described to yield the hybrid peptide (Ia).

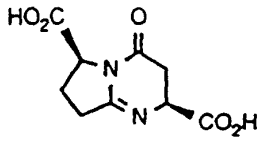
Scheme I


OMe
1. (COCl)2
2. KO-tBu, tBuOH
MeO
CO2H
(88%)
1
1. K, tBuOH, NH3 (l)
2. MeI
3. Hg(NO3)2
(53%)
CO2-tBu
2
I2, N2O4
CO2Me
3
NaOAc
(56% 2 steps)
4
Et3N
(66%)
H2 (50 psi)
Raney Ni
(79%)
5
1. KH
2. Boc-ON
(78%)
6
7
1. MeI
2. LiHMDS, HMPA, -78° (76%)
8
cis/trans
1. LiOH
2. Separate cis / trans
9-cis
(high Rf, 54%)
9-trans
(low Rf, 35%)
Val-OBn, PyBroP, DIEA, CH2Cl2 (78%)
10L
10D
1. H2, Pd/C (100%)
2. HPLC separation
11L
11D
1. Peptide coupling
2. Deprotection
Ia

In another aspect of the invention, hybrid peptide (IIa) is produced, containing helix inducer (IIb) replacing the N-terminal DG in the sequence of the TAM Mimic peptide TAM-1 (see Section 6.2).



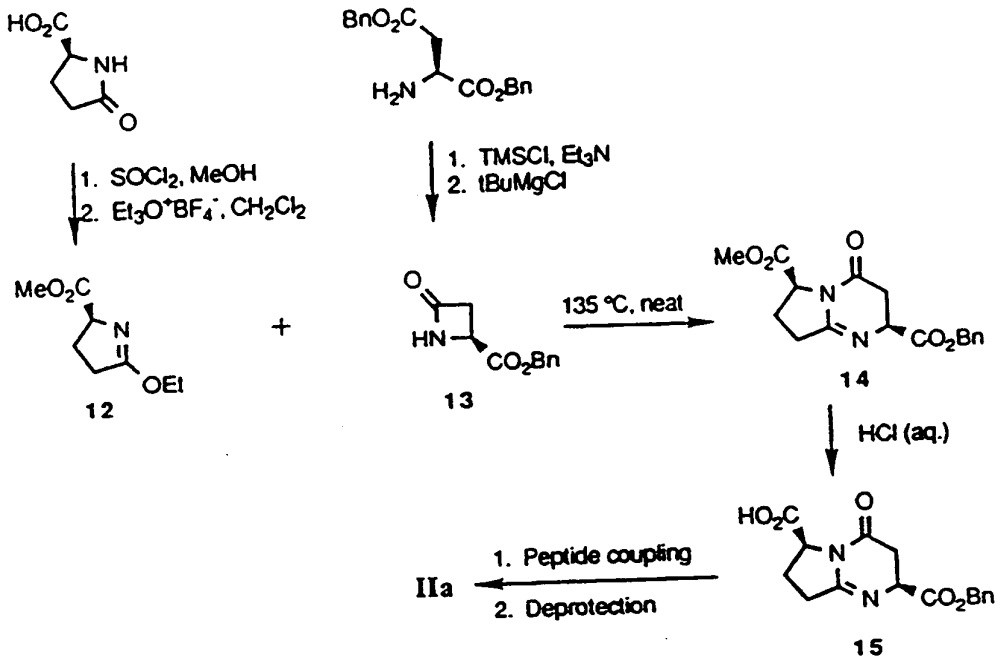
IIa



IIb

By way of example, the N-terminal helix inducer IIb can be synthesized from L-aspartic acid and L-pyroglutamic acid, according to Scheme II. Thus, L-pyroglutamic acid was first protected as its methyl ester by treatment with thionyl chloride in methanol at 0°C to room temperature (r.t.) for 20 h and then treated with triethyloxonium tetrafluoroborate in dichloromethane at r.t. for 2 d to give the imino ether (12). Reaction of the imino ether (12) with a 4-substituted-2-azetidinone (13) at 135°C neat for 4 h provided the desired hexahydro-pyrrolopyrimidine cis-dicarboxylic acid ester (14). The azetidinone (14) was formed from treatment of L-aspartic acid dibenzyl ester with trimethylsilyl chloride and triethylamine at 0°C for 1 h followed by tert-butyl magnesium chloride treatment for 20 h. Deprotection of the methyl ester of (14) by treatment with 10% aqueous HCl at 50°C is expected to give the free acid (15), which is coupled to TAM peptide 17-mer followed by removal of side chain protecting groups to provide the desired hybrid peptide mimic (IIa).

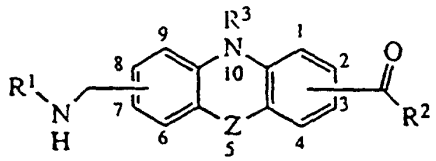
Scheme II


HO2C
NH
O
1. SOCl2, MeOH
2. Et3O+BF4-, CH2Cl2
MeO2C
N
OEt
12
+
BnO2C
H2N
CO2Bn
1. TMSCl, Et3N
2. tBuMgCl
O
HN
CO2Bn
13
135 °C, neat
MeO2C
O
N
N
CO2Bn
14
HCl (aq.)
HO2C
O
N
N
CO2Bn
15
1. Peptide coupling
2. Deprotection
IIa

5.3. HYBRID MOLECULES WITH INTERNAL HELIX REPLACEMENTS

TAM Mimics of the invention also include hybrid molecules with helix replacements to achieve gradual reduction of the peptide character of the molecules. In a preferred aspect, peptide hybrids of the invention are TAM Mimic peptides in which the peptide sequence corresponding to amino acid numbers 16-22 in the consensus sequence (SEQ ID NO:1) (see *e.g.* Fig. 3) are replaced by a phenothiazine ring structure.

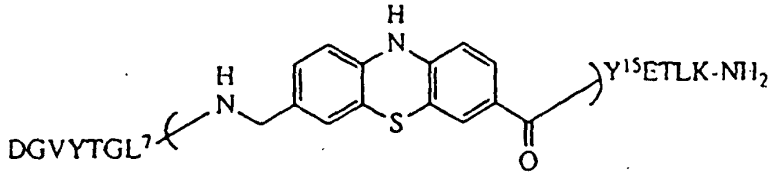
Compounds of the invention thus include peptide-like hybrid molecules of the formula (V) where R^1 is a sequence of at least 4, and is preferably 7, amino acids, wherein R^1 comprises a C-terminal leucine, and a tyrosine in the fourth position counting from a C to N-terminal direction. Thus R^1 corresponds to a portion of SEQ ID NO:1, amino-terminal to SEQ ID NO:1 residue number 16, except that the amino acid corresponding to residue number 9 of SEQ ID NO:1 can be any amino acid. In a specific but non-limiting aspect, R^1 has a sequence in the range of 4-25 amino acids. R^2 is a sequence of at least 4 amino acids, and is most preferably 5 amino acids, comprising an amino-terminal Tyr, and Leu or Ile in the fourth position counting in the N- to C-terminal direction. In a specific but non-limiting aspect, R^2 has a sequence in the range of 4-25 amino acids, and preferably 4-6 amino acids. R^3 is Hydrogen or C_1-C_6 alkyl. Z is S, SO, or SO_2.



(V)

A computer-generated drawing of a compound of formula (V) is shown in Figure 5.

A specific example of compound (V) is compound (Va):



(Va)

(replacing STRNQET in Seq. ID No. 13)

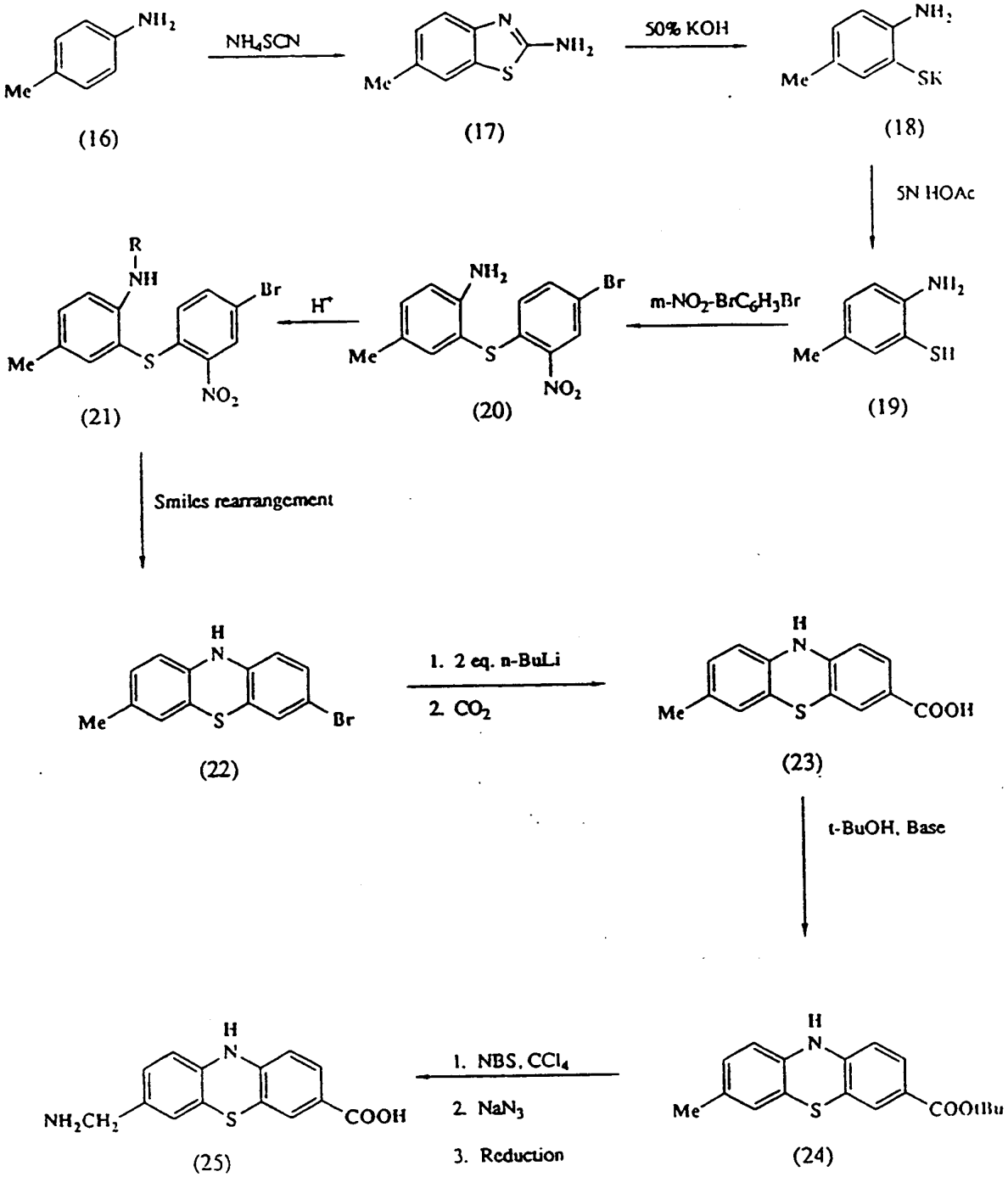
In specific embodiments, TAM Mimics of the invention which are peptide hybrids and which mimic the FcεRI γ TAM, are of structure (V) in which R^3 is H; Z is S; R^2 is YETLK-NH_2 (part of SEQ ID NO:17); and R^1 is DGVYTGL (part of SEQ ID NO:17), QKVYDKL (part of SEQ ID NO:19), or QKVYCKL (part of SEQ ID NO:20).

Exemplary compounds of Formula (V) which may be synthesized by the methods described herein are shown in Figure 4.

Compounds of Formula (V) wherein R^3 is hydrogen; Z is S, SO, or SO_2; and disubstitution occurs at the 3 and 7 positions of the phenothiazine ring may be prepared according to Scheme III. Condensation of 4-methyl aniline (16) (1 equiv.) (R. Adams, 1957, Org. Reactions, 3:240D) with ammonium thiocyanate under standard conditions [NH_4SCN (2 equiv.) in HOAc, Br_2 in HOAc (1 equiv., 8-10°C, 1 h] yields the corresponding 5-methyl-2-amino benzothiazole (17). Hydrolysis of (17) using 50% aqueous potassium hydroxide affords the

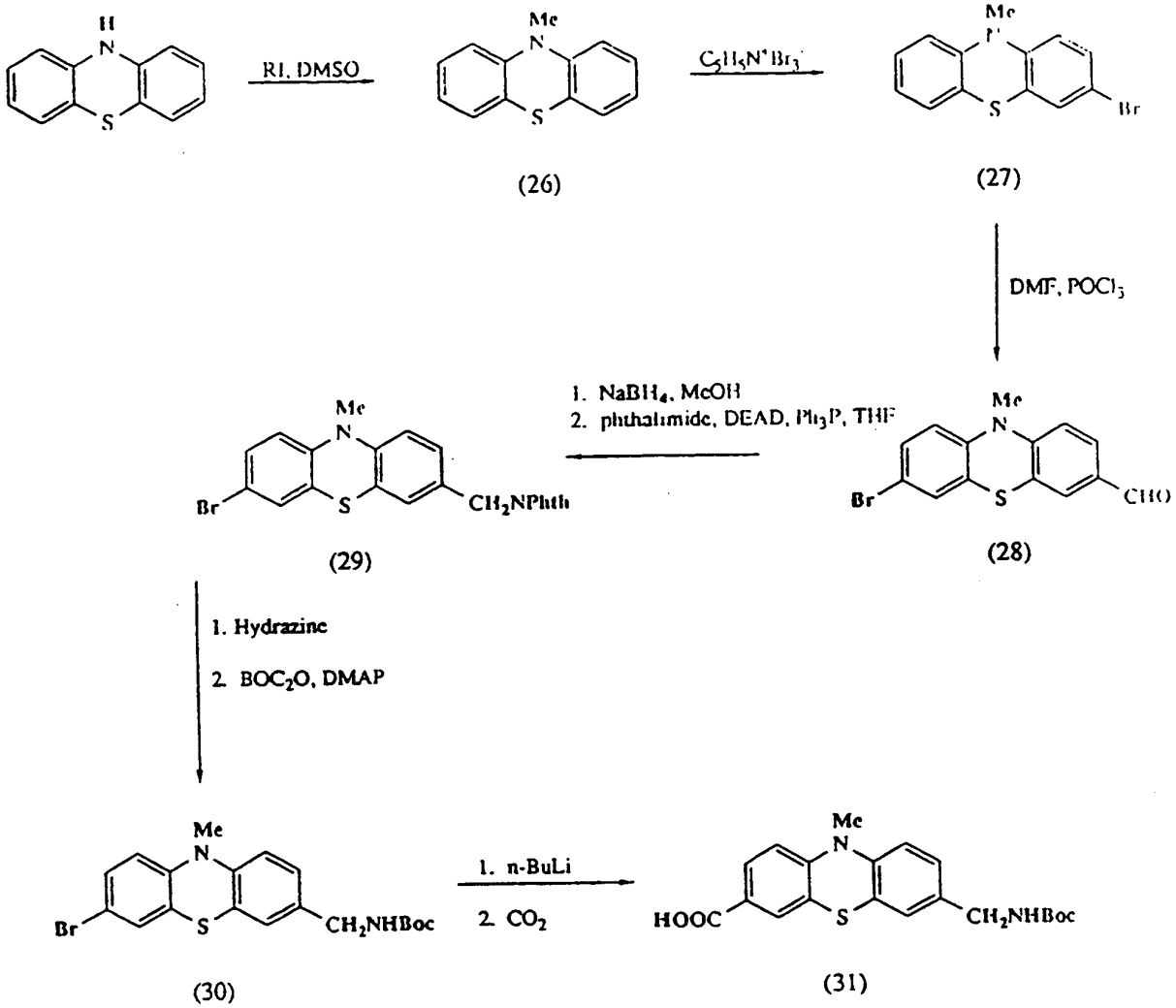
potassium salt of 2-amino-4-methyl thiophenol (18). Protonation of (18) with five normal acetic acid affords the thiol (19) which when condensed with meta-nitrobromobenzene in ethanol yields the sulfide (20) (Mital, 1969, J. Chem. Soc. (C), 2148). Formylation of the amino function with formic acid or acetylation of the amine with acetic anhydride yields the formamide or acetamide derivative (21), respectively (Jain et al., 1991, Indian J. Chem. 9:1236). Smiles rearrangement (Jain et al., 1991, Indian J. Chem. 9:1236) of (21) leads to 3-bromo-7-methyl phenothiazine (22). Transmetallation of (22) with 2 equivalents of n-Butyllithium at -78°C in tetrahydrofuran followed by quenching with carbon dioxide yields the carboxylic acid (23). Carboxylic acid (23) is converted to its tert-butyl ester (24) via standard conditions (Armstrong et al., 1988, Tet. Lett. 29(20):2483). Benzylic bromination of the methyl group of (24) with N-bromosuccinimide at reflux in carbon tetrachloride affords the benzyl bromide which is treated with sodium azide to give the corresponding azido derivative. Reduction of the azide under standard conditions yields the amino ester (25). Peptide coupling with the amino end of (25) followed by conversion of the tert-butyl ester to a carboxylic acid followed by a second peptide coupling with the carboxyl end of (25) yields the desired peptide-like hybrid molecules.

Scheme III


NH2
Me
NH4SCN
N
NH2
S
50% KOH
NH2
SK
(16)
(17)
(18)
5N HOAc
R
NH
Br
H+
NH2
m-NO2-BrC6H3Br
SH
NO2
(21)
(20)
(19)
Smiles rearrangement
H
N
1. 2 eq. n-BuLi
2. CO2
COOH
(22)
(23)
t-BuOH, Base
NH2CH2
1. NBS, CCl4
2. NaN3
3. Reduction
COOtBu
(25)
(24)

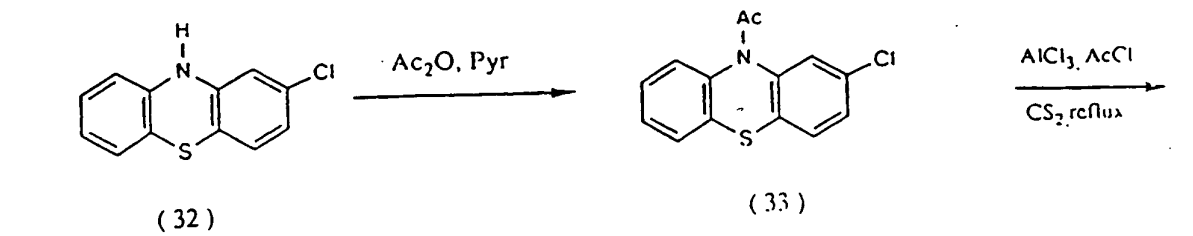
Compounds wherein R^1 is alkyl; Z is S, SO, or SO_2; and disubstitution occurs at the 3 and 7 positions of the phenothiazine ring may be prepared according to Scheme IV. Treatment of phenothiazine with iodomethane in dimethylsulfoxide yields the N-methylated derivative (26). Reaction of (26) with pyridinium bromide perbromide yields the 3-bromo derivative (27) (L. R. Biehl et al., 1974, J. Heterocyclic Chem. 11:247) which is subsequently formylated with dimethylformamide and phosphorus oxychloride yielding the aldehyde (28). Reduction of the aldehyde with sodium borohydride in methanol affords the corresponding benzyl alcohol. The benzyl alcohol is converted to the phthalimido derivative (29) (Bose et al., 1973, Tet. Lett. 1619) to afford the protected benzyl amine (29) (Bose et al., 1973, Tet. Lett. 1619). The phthalimide group is removed with hydrazine producing the free benzyl amine which is subsequently protected as its BOC (tert-butoxycarbonyl) derivative (30) by reaction with 2-(tert-butoxycarbonyloxyamino)-2-phenylacetonitrile. Metallation of (30) with n-butyllithium at -78°C followed by quenching with carbon dioxide produces the carboxylic acid (31). Peptide coupling with the carboxyl end of (31) followed by deprotection of the amine followed by a second peptide coupling with the amino end of (31) yields the desired peptide-like hybrid molecules.

Scheme IV

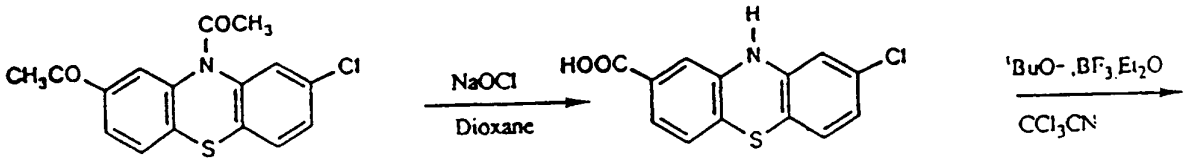

RI, DMSO
$C_5H_5N^+Br_3^-$
(26)
(27)
DMF, $POCl_3$
1. $NaBH_4$, MeOH
2. phthalimide, DEAD, Ph_3P, THF
(28)
(29)
1. Hydrazine
2. BOC_2O, DMAP
1. n-BuLi
2. CO_2
(30)
(31)

Compounds where R^1 is alkyl; Z is S, SO, or SO_2; and disubstitution occurs at the 2 and 8 positions of the phenothiazine ring may b prepared according to Scheme V. 2-Chloro-phenothiazine (32) (available from Aldrich Chemical Co.) is acylated with acetic anhydride and pyridine to give (33). Freidel-Crafts acylation of ester (33) with aluminum chloride and acetyl chloride in carbon disulfide at reflux affords the N-acetyl-2-carbmethoxy-8-acetyl phenothiazine (34). The acetyl moiety of compound (34) is selectively converted to a carboxylate with sodium hypochlorite in dioxane with concomitant removal of the N-acetyl group (Baltzy et al., 1946, J. Amer. Chem. Soc. 68:2673) to yield the 2-carbmethoxy-8-carboxy-phenothiazine (35). Esterification of the carboxyl moiety of (35) with tert-butyl 2,2,2-trichloroacetimidate and boron trifluoride etherate produces the diester (36). N-methylation of (36) with iodomethane in dimethylsulfoxide yields the N-methyl phenothiazine (37). (37) is treated with n-butyl lithium at -78°C in tetrahydrofuran to produce a metallated intermediate which is quenched with α-bromo-α'-phthalimido methane to give (38). Hydrazinolysis of (38) yields the desired amino ester (39). Peptide coupling with the amino end of (39) followed by conversion of the tert-butyl ester to a carboxylic acid followed by a second peptide coupling with the carboxyl end of (39) yields the desired peptide-like hybrid molecules.

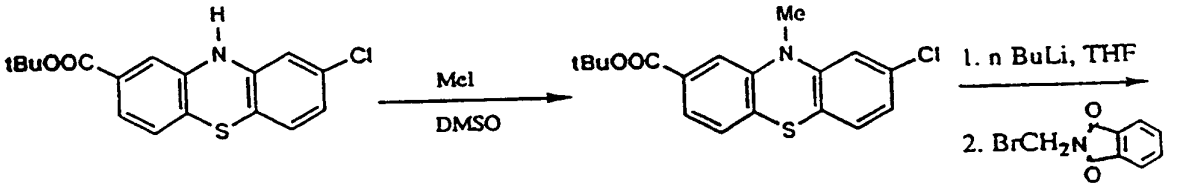
Scheme V



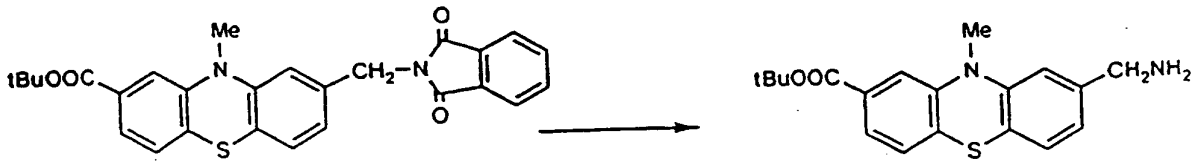
(32) (33)



(34) (35)



(36) (37)



(38) (39)

Schemes III, IV, and V, described *supra*, have been carried out as described. Particular compounds found in Schemes III, IV, and V have been synthesized according to the following protocols, presented by way of example:

Preparation of Compound 23

To a well stirred solution of 3-bromo-7methyl phenothiazine (1 equiv.) in 10-20 ml THF at -78°C, add η-BuLi (2.5 M solution;, 2 equiv.) slowly. After 30 min at -78°C, pass dry CO_2 gas through the reaction mixture for 15-20 min. Allow the reaction to come to r.t., quench it with water. Remove excess THF *in vacuo*. To the residue add a solution of sodium bicarbonate (1-2 equiv.). Wash the aqueous layer with ether (2 x 10 ml). After cooling the aqueous layer in ice, acidify with 5 N HCl to pH 2. Collect the crystals by filtration. Wash the crystals with water (10 ml). Dry in air. Recrystallize the acid from hot alcohol.

Preparation of Compound 28

This compound was prepared following the procedure for the preparation of indole 3-aldehyde from Indole Org. Synthesis Coll. Vol. 4, 539 (1972).

To dimethylformamide (DMF) (7.5 ml) cooled in ice was added freshly distilled $POCl_3$ (3.22 g, 21 mmol) and this Vilsmeier complex was stirred at 0°C in an atmosphere of N_2 for another 30 minutes. Then the 2-bromo-10-methyl phenothiazine (3.055 g, 10.55 mmol) was added in one lot followed by 2.5 ml DMF to rinse the side of the flask. After stirring at r.t. for 1 h (by which time the solid gets wetted by DMF and assumes a purple color), the flask was filled with a reflux condenser and heated on an oil bath at 90°C for

16 h. After 16 h, the whole mixture was poured over crushed ice (~ 250 g) and stirred for 20 min. Solid ammonium acetate was then added and the pH of the aqueous layer was checked from time to time. When the pH of the solution was ~ 7-8, the aqueous layer was repeatedly extracted with dichloromethane (3 x 75 ml). The dark yellow dichloromethane phase was washed (H_2O, 50 ml), dried (Na_2SO_4) and evaporated to leave a honey colored gum (3.58 g) which when chromatographed on a column of Silica gave yellow crystals of the bromoaldehyde (3.26 g, 97%) which was crystallized from hexane/EtoAC. M.p. 125°C.

Preparation of Compound 29

To a well stirred solution of the bromoaldehyde (28) (4 g; 0.0125 mol) in MeOH (720 ml) was added $NaBH_4$ (400 mg) in small portions in such a way that the reaction was not too vigorous. After 45 min, the reaction mixture was stirred for a further 30 min and the MeOH was evaporated *in vacuo*. To the residue was added water (25 ml) and repeatedly extracted with dichloromethane (25 ml x 5). The dichloromethane phase was washed (sat. saline, 25 ml), dried (Na_2SO_4) and evaporated to give a white solid, m.p. 114°C and was used as such for the next step without further purification and after drying over P_2O_5 for 24 h (4.011 g; ~ 98%). The next step was carried out as described (Bose et al., 1973, Tet. Lett. 1619; Bose et al., 1973, Tet. Lett. 3937).

Preparation of Compound 30

Part A

To a solution of phthalimido derivative (29) (400 mg; 0.888 mmol) in THF/EtOH (7/14 ml) was added anhydrous hydrazine (0.5 ml, a known excess) and this

mix was heated to reflux on an oil bath at 90°C for 16-18 h. The THF and EtOH were removed *in vacuo*. To the residue was added dichloromethane (30 ml) and filtered to remove the phthaloyl hydrazide. The residue was washed twice (5 ml each) with dichloromethane. The combined dichloromethane phase was concentrated *in vacuo* to give a yellow oil (292 mg).

Part B

To a solution of the amine (300 mg; 0.9375 mmol), BOC_2O (245.25 mg; 1.125 mmol), DMAP (30 mg) in THF (10 ml) in an atmosphere of N_2 with stirring was added Et_3N (114.5 mg; 1.125 mmol). This reaction mixture was allowed to stir at r.t. for 16-18 h after which THF was removed *in vacuo*. To the residue was added water (15 ml) and repeatedly extracted with dichloromethane (15 ml x 3). The combined dichloromethane phase was washed (H_2O, 10 ml), dried (Na_2SO_4) and evaporated to give a pale yellow gum (552 mg) which was purified by column chromatography on Silica gel using hexane-ethyl acetate 5-7% to give a white microcrystalline powder, m.p. 172°C (306 mg; 78%).

Preparation of Compound 31

To a well cooled solution of the bromo derivative (30) (100 mg; 0.2381 mmol) in THF (5 ml) at -78°C was added η-BuLi (2.5 M in hexane; 0.19 ml; 0.4762 mmol; 2 equiv.). The solution turned yellow immediately and this was allowed to stir at -78°C for 10 min (care should be taken to keep the temperature of the anion below -40°C as this will deprotect the BOC group on the amine). Dry CO_2 gas was passed through this solution carefully for 15 min and the

solution was allowed to come to r.t. The reaction was quenched with crushed ice. THF was removed *in vacuo*. A saturated solution of $NaHCO_3$ (5 ml) was added to the aqueous residue and washed with ether (2 x 5 ml). The aqueous layer was cooled in ice and acidified with conc. HCl to pH 2. The yellow precipitate was collected and washed with chilled water (2 x 5 ml), giving a yellow microcrystalline powder (66.17 mg; 72%).

5.4. CHARACTERIZATION OF TAM MIMICS

In a preferred although optional aspect, TAM Mimics are further characterized as described below prior to *in vitro* and *in vivo* testing of inhibitory/therapeutic activity. The conformational preferences and membrane-penetrating properties of the TAM Mimic peptides can be investigated both theoretically and experimentally.

Membrane-penetrating properties of a TAM Mimic peptide can be predicted based on four parameters (Schwyzer et al., 1986, Helvetica Chimica Acta 69:1789-1797): (i) the free energy of hydrophobic association, (ii) the molecular amphiphilic moment; (iii) the electric dipole of the peptide; and (iv) the net charge of the peptide.

These four factors can be combined to predict the energy of association for a peptide segment in a particular orientation with a lipid bilayer possessing known charge characteristics. By searching for the minimum energy conformation, it is possible to predict the length of helix formed on association, and its orientation with respect to the bilayer surface. This approach can be used to predict the effect of sequence modification on the lipid

bilayer binding properties of a series of peptides. These predictions can be tested as described below.

The lipid-induced helix-forming potential of a TAM Mimic can be assessed by CD (circular dichroism) spectroscopy in the presence of lipid-mimicking solvents (*e.g.*, trifluorethanol) and lipid vesicles. NMR (nuclear magnetic resonance) spectroscopy can be used to determine the three-dimensional structure of a TAM Mimic both in helix-promoting solvents and in the presence of liposomes.

NMR has been used extensively to study the three-dimensional structure of biologically active peptides in solution or bound to micelles or phospholipid vesicles. The use of NMR to study the solution conformation of proteins and peptides is well established (Wuthrich, K., 1986, *NMR of Proteins and Nucleic Acids*, John Wiley & Sons, Inc.) and similar NMR analyses have been applied to a number of peptide-bilayer systems.

The membrane transport properties of TAM Mimics can be studied by size exclusion chromatography. Lipid vesicles are prepared, incubated with TAM Mimics, then chromatographed to separate free TAM Mimic. The vesicle contents are then assayed to determine the amount of TAM Mimic present in the lipid vesicle fraction (determinable by HPLC or amino acid analysis). A fluorescence assay for peptide transport can be used; the assay uses a water-stable fluorogenic reagent that becomes trapped inside the vesicles during formation. This fluorogenic reagent reacts with TAM Mimic molecules that have crossed the lipid bilayer, producing a fluorescent derivative.

5.5. DEMONSTRATION OF THERAPEUTIC UTILITY

TAM Mimics are tested *in vitro* and then preferably *in vivo* for the desired therapeutic utility.

Any *in vitro* assay known in the art can be used to detect inhibition by a TAM Mimic of the invention. For example, in a preferred embodiment wherein a TAM Mimic inhibitory to mast cell or basophil activation is desired, a functional *in vitro* assay for mast cell or basophil degranulation is employed (see, *e.g.*, Section 8 *infra*, and Barsumian et al., 1981, Eur. J. Immunol. 11:317-323; Cunha-Melo et al., 1989, J. Immunol. 143:2617-2625). The release of histamine, the release of beta-hexosaminidase, the release of cytokines (*e.g.*, interleukins) and/or increased phosphatidylinositol hydrolysis or tyrosine phosphorylation can be detected in *in vitro* assays as indications of mast cell or basophil activation (see *e.g.*, Stephan et al., 1992, J. Biol. Chem. 267:5434). Histamine release can be assayed, for example, by commercially available radioimmunoassay (*e.g.*, AMAC Inc., Westbrook, Maine, Cat. No. 1302). Basophilic cell lines such as RBL-2H3 (Stephan et al., *supra*), KU812 (Matsson et al., 1989, Int. Arch. Allergy Appl. Immunol. 88:122-125; Valent et al., 1990, J. Immunol. 145:1885-1889), etc. can be employed in such *in vitro* assays. The release of histamine from basophils is an *in vitro* assay of immediate hypersensitivity (Ishizaka & Ishizaka, 1975, Prog. Allergy 19:60). Other specific examples of *in vitro* assays of activation of other cell types are as follows: Natural killer activation can be assayed, *e.g.*, by *in vitro* cytotoxicity assays measuring natural killer lysis of target cells such as K562 erythromyeloid leukemia cells. Macrophage activation can be assayed by

measuring macrophage phagocytic activity, induction of macrophage cytoxicity, or induction of macrophage Class II MHC cell surface expression, or by observing morphological changes associated with activation (see *e.g.*, Wright and Meyer, 1985, J. Exp. Med. 162:762-767; Fidalgo and Najjar, 1967, Biochemistry 6(11):3386-3392; U.S. Patent No. 5,049,659 dated September 17, 1991). T cell activation can be assayed by T cell proliferation *in vitro*, or by measuring expression of cell surface interleukin-2 receptor (IL-2R), which increases upon activation of T cells (Waldman et al., 1984, J. Exp. Med. 160:1450-1466). B cell activation can be assayed by measuring B cell proliferation *in vitro*.

TAM Mimics demonstrated to have the desired activity *in vitro* can be tested *in vivo* for the desired inhibitory activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. Suitable model systems are also used to demonstrate therapeutic utility (see *infra*).

For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. For example, an animal model system for rheumatoid arthritis is that consisting of animals of the autoimmune MRL/l mouse strain (Murphy, E.D. and Roths, J.B., 1978, in Genetic Control of Autoimmune Disease, Rose, N.R., *et al.*, eds., Elsevier/North-Holland, New York, pp. 207-219), that develop a spontaneous rheumatoid arthritis-like disease (Hang et al., 1982, J. Exp. Med. 155:1690-1701). There are numerous animal models of asthma that have been developed and can be used (for reviews, see Larson, 1991, "Experimental models of reversible airway

obstruction," in *The Lung: Scientific Foundations*, Crystal, West et al. (eds.), Raven Press, New York, pp. 953-965; Warner et al., 1990, Am. Rev. Respir. Dis. 141:253-257). Species used as animal models for asthma include mice, rats, guinea pigs, rabbits, ponies, dogs, sheep, and primates.

Many other *in vivo* models are available and can be used, *e.g.*, as described in the following references:

Cross et al. (1990) Homing to central nervous system vasculature by antigen-specific lymphocytes. I. Localization of ^{14}C-labeled cells during acute, chronic, and relapsing experimental allergic encephalomyelitis. Lab. Invest. 63:162-170.

Kakimoto et al. (1992) The effect of anti-adhesion molecule antibody on the development of collagen-induced arthritis. Cell. Immunol. 142:326-337.

Keffer et al. (1991) Transgenic mice expression human tumour necrosis factor: A predictive genetic model of arthritis. EMBO J. 10:4025-4031.

Koh et al. (1992) Less mortality but more relapses in experimental allergic encephalomyelitis in CD8$^{-/-}$ mice. Science 256:1210-1213.

Raine, C.S. (1991) Multiple sclerosis: a pivotal role for the T cell in lesion development. Neuropathol. Appl. Neurobiol. 17:265-274.

Shiozawa et al. (1992) Destructive arthritis without lymphocyte infiltration in H2-c-fos transgenic mice. J. Immunol. 148:3100-3104.

Thorbecke et al. (1992) Involvement of endogenous tumor necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice. Proc. Natl. Acad. Sci. USA 89:7375-7379.

5.6. THERAPEUTIC AND PROPHYLACTIC USES

The TAM Mimics have therapeutic and prophylactic utility in the modulation of functions mediated by MIRRs, in particular in diseases or disorders involving the immune system or inflammation (inflammatory and immune disorders). TAM Mimics which inhibit lymphocytes are important therapeutically, because lymphocytes initiate autoimmune and alloimmune diseases.

TAM Mimics which inhibit an immune or inflammatory response and thus are useful according to the invention are most preferably identified by use of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit activation of cells of the immune system assayed *in vitro*, or *in vivo* assays (see Section 5.5 *supra*). TAM Mimics which inhibit activation of T cells, B cells, and/or macrophages *in vitro* are preferred for treatment (or prevention) of immune disorders such as but not limited to autoimmune diseases and transplant rejection. TAM Mimics which inhibit activation of macrophages and/or natural killer cells *in vitro* are preferred for treatment (or prevention) of immune complex diseases such as but not limited to glomerulonephritis and other autoimmune diseases. TAM Mimics which inhibit activation of mast cells *in vitro* are preferred for treatment (or prevention) of type I allergic (IgE-mediated) reactions such as but not limited to asthma and allergic rhinitis. Further descriptions of diseases

and disorders subject to treatment with TAM Mimics are described below.

The invention provides methods of treating or preventing diseases and disorders associated with undesirable or inappropriate immune system activity or inflammation by administration to a subject of an effective amount of a TAM Mimic of the invention.

The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Diseases and disorders which can be treated by administration of a therapeutically effective amount of a TAM Mimic which inhibits the inflammatory/immune response include but are not limited to the following:

Inflammatory arthritis - *e.g.*, rheumatoid arthritis, seronegative spondyloarthritites (Behcets disease, Reiter's syndrome, etc.), juvenile rheumatoid arthritis, vasculitis, psoriatic arthritis, polydermatomyositis.

Systemic lupus erythematosus (SLE).

Asthma.

Inflammatory dermatoses - *e.g.*, psoriasis, dermatitis herpetiformis, eczema, necrotizing and cutaneous vasculitis, bullous diseases.

Inflammatory bowel disease - Crohn's disease and ulcerative colitis.

Tissue damage relating to tissue transplantation.

Other autoimmune disorders. In addition to the autoimmune disorders SLE and rheumatoid arthritis, disorders such as glomerulonephritis, juvenile onset diabetes, multiple sclerosis, allergic conditions, autoimmune thyroiditis, allograft rejection (*e.g.*,

rejection of transplanted organs such as kidney, heart, pancreas, bowel, or liver), and graft-versus-host disease can be treated.

In addition, other diseases and clinical correlates of undesirable inflammatory responses can be treated with inhibitor TAM Mimics of the invention, including but not limited to those associated with hemolytic anemia, blood transfusion, certain hematologic malignancies, scleroderma, atherosclerosis, cytokine-induced toxicity, necrotizing enterocolitis, granulocyte-transfusion-associated syndromes, Reynaud's syndrome, or other central nervous system inflammatory disorders.

Furthermore, in a preferred aspect of the invention, a TAM Mimic which inhibits mast cell activation is administered to treat (or prevent) a type I allergic reaction such as one or more of the following listed in Table 1 (see generally, Terr, 1987, in *Basic & Clinical Immunology*, 6th Ed., ch. 24, Stites et al. (eds), Appleton & Lange, Norwalk, Connecticut, pp. 435-456).

TABLE 1

Atopic Diseases

- allergic rhinitis (hay fever) - *e.g.*, due to pollens, fungal spores, dust, animal dander
- asthma
- atopic dermatitis
- allergic gastroenteropathy (due to ingested food)

Anaphylaxis (a systemic immediate hypersensitivity affecting multiple organs), *e.g.* due to drugs (proteins such as in vaccines, and nonproteins such as antibiotics, anesthetics, salicylates), foods, venom of stinging insects)

Urticaria or Angioedema (increased cutaneous vascular permeability)

5.7. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a TAM Mimic of the invention. In a preferred aspect, the TAM Mimic is purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a TAM Mimic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a TAM Mimic-encoding nucleic acid as part of a

retroviral or other vector, etc. Since preferred TAM Mimics of the invention are permeable to the cell membrane, a preferred mode of delivery is via pulmonary administration, as detailed more fully in Section 5.7.1 *infra*. However, methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The TAM Mimics may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. For TAM Mimics which are used for inhibition of mast cell activation, *e.g.*, for therapy of asthma or allergy, the preferred route of administration is nasal or via a bronchial aerosol.

In a specific embodiment, it may be desirable to administer the TAM Mimics of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (*e.g.*, for skin conditions such as psoriasis), by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically (or prophylactically) effective amount of a TAM Mimic, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline,

buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection

5.7.1. PULMONARY ADMINISTRATION

In a preferred embodiment of the invention, a TAM Mimic is administered by pulmonary administration. In particular, a bronchial aerosol is employed. This mode of administration is particularly preferred where the TAM Mimic inhibits mast cell activation and thus is useful in treating IgE-related disorders. Thus, pulmonary administration is preferred for treatment, *e.g.*, of allergy or asthma.

Pulmonary administration can be accomplished, for example, using any of various delivery devices known in the art (see *e.g.*, Newman, S.P., 1984, in *Aerosols and the Lung*, Clarke and Davia (eds.), Butterworths, London, England, pp. 197-224; PCT Publication No. WO 92/16192 dated October 1, 1992; PCT Publication No. WO 91/08760 dated June 27, 1991; NTIS Patent Application 7-504-047 filed April 3, 1990 by Roosdorp and Crystal), including but not limited to nebulizers, metered dose inhalers, and powder inhalers. Various delivery devices are commercially available and can be employed, *e.g.*, Ultravent nebulizer (Mallinckrodt, Inc., St. Louis, Missouri); Acorn II nebulizer (Marquest Medical Products, Englewood, Colorado), Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, North Carolina); Spinhaler powder inhaler (Fisons Corp., Bedford, Massachusetts). Such devices typically entail the use of formulations suitable for dispensing from such a device, in which a propellant material may be present.

Ultrasonic nebulizers tend to be more efficient than jet nebulizers in producing an aerosol of respirable size from a liquid (Smith and Spino, "Pharmacokinetics of Drugs in Cystic Fibrosis," Consensus Conference, Clinical Outcomes for Evaluation

of New CF Therapies, Rockville, Maryland, December 10-11, 1992, Cystic Fibrosis Foundation).

A nebulizer may be used to produce aerosol particles, or any of various physiologically acceptable inert gases may be used as an aerosolizing agent. Other components such as physiologically acceptable surfactants (*e.g.*, glycerides), excipients (*e.g.*, lactose), carriers, and diluents may also be included.

6. SYNTHESIS OF 19-MER TAM MIMICS

Five different 19-mer Tam Mimic peptides were synthesized, as described *infra*. The five peptides had the following sequences, and were termed respectively TAM-1, TAM-2, TAM-4, TAM-5, and TAM-6:

Ac-D G V Y T G L S T R N Q E T Y E T L K-NH_2 (SEQ ID NO:19);

Ac-Q K V Y D K L L K R N Q E T Y E T L K-NH_2 (SEQ ID NO:20);

Ac-Q K V Y C K L L C R N Q E L Y E T L K-NH_2 (SEQ ID NO:22);

Ac-D G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:23); and

Ac-N G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:24).

6.1. SYNTHESIS OF PEPTIDES

6.1.1. GENERAL PROCEDURES FOR SOLID PHASE SYNTHESIS

Peptides were synthesized on an Applied Biosystems Inc. ("ABI") model 431A automated peptide synthesizer using the "Fastmoc" synthesis protocol supplied by ABI, which uses

2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate ("HBTU") (R. Knorr et al., 1989, Tet. Lett., 30:1927) as coupling agent. Syntheses were carried out on 0.25 mmol of commercially available 4-(2',4'-dimethoxyphenyl-(9-fluorenyl-methoxycarbonyl)-aminomethyl)-phenoxy polystyrene resin ("Rink resin" from Advanced ChemTech) (H. Rink, 1987, Tet. Lett. 28:3787). Fmoc amino acids (1 mmol) were coupled according to the Fastmoc protocol. The following side chain protected Fmoc amino acid derivatives were used: FmocArg(Pmc)OH; FmocAsn(Mbh)OH; FmocAsp(^{t}Bu)OH; FmocCys(Acm)OH; FmocGlu(^{t}Bu)OH; FmocGln(Mbh)OH; FmocHis(Tr)OH; FmocLys(Boc)OH; FmocSer(^{t}Bu)OH; FmocThr(^{t}Bu)OH; FmocTyr(^{t}Bu)OH. [Abbreviations: Acm, acetamidomethyl; Boc, tert-butoxycarbonyl; ^{t}Bu, tert-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; Mbh, 4,4'-dimethoxybenzhydryl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tr, trityl].

Synthesis is carried out using N-methylpyrrolidone (NMP) as solvent, with HBTU dissolved in N,N-dimethylformamide (DMF). Deprotection of the Fmoc group is effected using ca. 20% piperidine in NMP. At the end of each synthesis the amount of peptide present is assayed by ultraviolet spectroscopy. A sample of dry peptide resin (ca. 3-10 mg) is weighed, then 20% piperidine in DMA (10 mL) is added. After 30 min sonication, the UV (ultraviolet) absorbance of the dibenzofulvene-piperidine adduct (formed by cleavage of the N-terminal Fmoc group) is recorded at 301 nm. Peptide substitution (in mmol g^{-1}) can be calculated according to the equation:

$$\text{substitution} = \frac{A \times v}{7800 \times w} \times 1000$$

where A is the absorbance at 301 nm, v is the volume of 20% piperidine in DMA (in mL), 7800 is the extinction coefficient (in $mol^{-1}dm^3cm^{-1}$) of the dibenzofulvene-piperidine adduct, and w is the weight of the peptide-resin sample (in mg).

Finally, the N-terminal Fmoc group is cleaved using 20% piperidine in DMA, then acetylated using acetic anhydride and pyridine in DMA. The peptide resin is thoroughly washed with DMA, CH_2Cl_2 and finally diethyl ether.

6.1.2. CLEAVAGE AND DEPROTECTION -- GENERAL PROCEDURE

The air-dried peptide resin is treated with ethylmethyl-sulfide (EtSMe), ethanedithiol (EDT), and thioanisole (PhSMe) for approximately 20 min. prior to addition of 95% aqueous trifluoracetic acid (TFA). A total volume of ca. 50 mL of these reagents are used per gram of peptide-resin. The following ratio is used: TFA : EtSMe : EDT : PhSme (10 : 0.5 : 0.5 : 0.5). The mixture is stirred for 3 h at room temperature under an atmosphere of N_2. The mixture is filtered and the resin washed with TFA (2 x 3 mL). The combined filtrate is evaporated *in vacuo*, and anhydrous diethyl ether added to the yellow/orange residue. The resulting white precipitate is isolated by filtration.

6.2. TAM-1 PEPTIDE

The following 19-mer TAM Mimic peptide, termed "TAM-1", having the TAM native sequence shown was synthesized:

Ac-D G V Y T G L S T R N Q E T Y E T L K-NH_2

(SEQ ID NO:19).

6.2.1. SYNTHESIS

Synth sis was carried out according to the procedure detailed in Section 6.1.1. UV analysis following the final coupling of FmocAsp(^{t}Bu)OH to the peptide-resin revealed a substitution of 0.147 mmol g^{-1} (0.194 mmol g^{-1} is theoretically predicted, based on an initial resin substitution of 0.62 mmol g^{-1}). The peptide-resin was subsequently acetylated (see Section 6.1.1) and 600 mg of the peptide-resin was cleaved and deprotected in accordance with the procedure described in Section 6.1.2.

6.2.2. PURIFICATION OF Ac-(TAM-1)-NH_2

The crude Ac-(TAM-1)-NH_2 (276 mg) was partially purified by gel filtration in 10% aqueous acetic acid on Sephadex G-10 (column 2.5 cm x 40 cm). Product-containing fractions were pooled and lyophilized to give 152 mg material which was further purified by reversed phase HPLC on a Vydac C18 column (25 x 250 mm) to give 52 mg of >90% pure peptide.

6.3. TAM-2 PEPTIDE

The following TAM Mimic peptide, termed TAM-2, was synthesized:
Ac-Q K V Y D K L L K R N Q E T Y E T L K-NH_2
(SEQ ID NO:20). Non-consensus amino acids were changed relative to the TAM-1 peptide sequence, with a view toward achieving a higher degree of helicity.

6.3.1. SYNTHESIS

Synthesis was carried out by the methods described in Section 6.1.1.

6.4. TAM-4 PEPTIDE

The following TAM Mimic peptide, termed TAM-4, was synthesized:

Ac-Q K V Y C K L L C R N Q E L Y E T L K-NH_2 (SEQ ID NO:22). The peptide contained two cysteine residues linked via a disulfide bridge to help lock the peptide into the presumed desired conformation.

6.4.1. SYNTHESIS

Synthesis was carried out according to the methods described in Section 6.1.1, then 766 mg of the peptide-resin was cleaved and deprotected as described in Section 6.1.2 to give 385 mg of crude peptide.

6.4.2. PURIFICATION, CYCLIZATION AND ISOLATION OF TAM-4 PEPTIDE

The crude peptide (385 mg) was initially partially purified by gel filtration on Sephadex G-10 eluted with 10% aqueous acetic acid to give 300 mg material. Peptide (73 mg) was dissolved in degassed 50% aqueous acetic acid (2.5 mL) and mercuric acetate (91 mg) in 50% aqueous acetic acid (1.0 mL) was added. The mixture was stirred under N_2 for 3 h, then β-mercaptoethanol (300 μL) was added and the mixture stirred overnight. The grey precipitate was separated by centrifugation and the supernatant desalted on a Sephadex G-10 column eluted with 10% aqueous acetic acid under an atmosphere of N_2. Desired fractions were pooled and lyophilized. The peptide (62 mg) was reduced by dissolution in 6 M urea/0.2 M tris(trihydroxymethyl)aminomethane-HCl buffer pH 8 (3 mL), followed by addition of dithiothreitol (40 mg) in the same buffer (1 mL). The mixture was stirred in the dark for 3 h under an atmosphere of N_2. The sample was desalted on a Sephadex G-10 column eluted with 1 M

NH_4HCO_3 pH 8 buffer under an atmosphere of N_2. Relevant column fractions were combined, diluted to a total volume of 620 mL (*i.e.*, 0.1 mg ml^{-1}) with 1 M NH_4HCO_3 and stirred vigorously in air for 66 h. The sample was then lyophilized and subsequently desalted on Sephadex G-10 eluted with 10% aqueous AcOH to give 35 mg of product. Further purification was carried out by HPLC on a Kromasil C8 100 Å column (20 x 250 mm, eluted with a gradient of 30-50% MeCN in H_2O (0.1% TFA). Purified product was 10.5 mg.

6.5. TAM-5 PEPTIDE

The following TAM Mimic peptide, termed TAM-5 was synthesized:

Ac-D G V Y T G L S T R N Q K T Y K T L K-NH_2

(SEQ ID NO:23). In this sequence the two glutamate residues of the native sequence (TAM-1) are replaced by lysine.

6.5.1. SYNTHESIS

Synthesis was carried out as described in Section 6.1.1. UV analysis of the peptide-resin following coupling of FmocAsp(^{t}Bu) revealed a substitution of 0.138 mmol g^{-1} (compared to 0.191 mmol g^{-1} predicted from an initial resin substitution of 0.62 mmol g^{-1}). The peptide-resin was acetylated (Section 6.1.1), then 466 mg was cleaved and deprotected as described in Section 6.1.2 to afford 400 mg crude material. Purification of 49 mg crude peptide on reversed-phase HPLC (Kromasil C8, 100 Å, 10 μ particle size, 20 x 250 mm column) using a gradient of MeCN in H_2O (0.1% TFA) gave 21 mg of the desired product.

6.6. TAM-6 PEPTIDE

The following TAM Mimic peptide, termed TAM-6 was synthesized:

Ac-N G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:24). In this sequence the aspartate residue of the native sequence (TAM-1) is replaced by asparagine, and the two glutamate residues are replaced by lysine; thus all acidic amino acids are substituted.

6.6.1. SYNTHESIS

Synthesis was carried out as described in Section 6.1.1. UV analysis of the peptide resin following coupling of FmocAsn(Mbh)OH revealed a substitution of 0.109 mmol g^{-1} (compared to a predicted value of 0.185 mmol g^{-1} from an initial resin substitution of 0.62 mmol g^{-1}). The peptide-resin was acetylated (Section 6.1.1), then 358 mg peptide-resin was cleaved and deprotected as described in Section 6.1.2 to give 199 mg of crude product. Crude peptide (153 mg) was purified by reversed-phase HPLC on a column packed with Kromasil C8, 100 Å, 10 μ particle size (20 x 250 mm) eluted with a gradient of MeCN in H_2O) (0.1% TFA) to give 38 mg of the desired peptide, TAM-6.

7. SECONDARY STRUCTURE DETERMINATION OF TAM-1

The synthesized and purified TAM-1 (see Section 6.1) was subjected to nuclear magnetic resonance (NMR) spectroscopy, which allowed the determination of its secondary structure and appropriate templates for helix nucleation in the formation of peptide hybrids (see Section 5.2).

To carry out the NMR spectroscopy, the purified TAM-1 was prepared both as a 3 mM solution in

30% (v/v) deuterated trifluoroethanol (30% TFE) and as a 3 mM solution in the presence of deuterated dodecylphosphatidylcholine (DPC) micelles. NMR spectra were acquired using 600 MH_z NMR spectrometers (Bruker AMX600). A series of two-dimensional spectra were obtained for each sample (DQF-COSY, HOHAHA and NOESY experiments), and the NMR spectra for both samples were assigned using standard sequential assignment methodologies (Wuthrich K., 1986, NMR of Proteins and Nucleic Acids, John Wiley and Sons, Inc.).

The NMR analysis of the secondary structure of the TAM-1 peptides was based on an analysis of the relative intensities of interproton NOE data (Wuthrich K., 1986, NMR of Proteins and Nucleic Acids, John Wiley and Sons, Inc.) and a chemical shift index analysis (Wishart et al., 1992, Biochem. 31:1647-1651).

For both samples of TAM-1 sequential and medium range NOE peak intensities indicated that a helical structure is present in the C-terminal portion of the molecule.

The results of the chemical shift index analysis were as follows:

Residue	30% TFE Score	DPC Score
Asp 1	-	-
Gly 2	0	0
Val 3	0	0
Tyr 4	0	0
Thr 5	-1	-1
Gly 6	-1	-1
Leu 7	+1	+1
Ser 8	-1	-1
Thr 9	-1	-1
Arg 10	-1	-1
Asn 11	-1	-1
Gln 12	-1	-1
Glu 13	-1	-1
Thr 14	-1	-1
Tyr 15	-1	-1
Glu 16	-1	-1
Thr 17	-1	-1
Leu 18	0	0
Lys 19	-	-

An extended sequence of -1 scores in the chemical shift index analysis implies that a helical conformation is present in the peptide. The chemical shift index analysis results above are both self-consistent and consistent with the analysis of the NOE data. Taken together the analyses imply that the peptide exists in a predominantly helical conformation in both solvent systems. Such a helical conformation contains three turns, with the conserved Tyr and Leu residues from the motif stacked on the same side of the molecule. We propose that the face

of the helix that contains the conserved amino acids is the functionally important side and that the other side of the helix can accommodate structural changes.

8. MAST CELL/BASOPHIL DEGRANULATION ASSAYS

8.1. INTACT CELL ASSAYS

The release of β-hexosaminidase from basophils is measured as an *in vitro* assay of basophil activation. A cell line, RBL-2H3 (from the laboratory of Dr. Siraganian, National Institutes of Health), of rat basophilic leukemia (RBL) cells is used in such an *in vitro* assay, according to the protocol described below (see also Barsumian et al., 1981, Eur. J. Immunol. 11:317-323). The percentage of β-hexosaminidase in cells that is released into the cell medium is determined, and is a measure of the activation of the RBL-2H3 cells.

8.1.1. ACTIVATION OF 2BL-2H3 CELLS

Materials

- RBL medium containing 2% fetal bovine serum (FBS)

  100 ml E-MEM
  2 ml FBS
  1 ml glutamine
  1.2 ml penicillin-streptomycin

  filter sterilize into sterile bottles or tissue culture flasks and store at 4°C

- 10x PIPES buffer

  75.6 g PIPES (0.25 M)
  69.2 g NaCl (1.2 M)

3.72 g KCl (0.05 M)
43 ml of 10 N NaOH (0.4 M)
Add water to 1000 ml

filter sterilize into sterile bottles or tissue culture flasks and store at 4°C

- 100x calcium chloride

1.47 g $CaCl_2$-$2H_2O$ (100 mM)
Add water to 100 ml

filter sterilize into sterile bottles or tissue culture flasks and store at 4°C

- 2x-IgE (anti DNP-IgE mouse monoclonal antibody; Zymed) in regular RBL medium containing 15% FBS

Need 0.5 ml at 2-times an appropriate final dilution for each 16 mm well to be seeded with cells

- 1x PIPES+glucose+calcium (prepare fresh on second day of assay)

10 ml of 10X PIPES
0.1 g glucose (dextrose) (5.6 Mm)
0.1 g bovine serum albumin (BSA)
1 ml of 100x calcium chloride

Add water to 90 ml; pH to 7.4; milli-Q water to 100 ml

Method

- Remove RBL cells from flasks by trypsin-EDTA treatment (see protocol *infra*). Plan to use 1×10^5 cells/16 mm well to be seeded; harvest extra cells to allow for losses during centrifugation.

- Centrifuge the RBL cells and resuspend in 5 ml of regular RBL medium containing 15% FBS. Count the cells and dilute them to 2×10^5 cells/ml in the same medium.

- For each well to be seeded, mix 0.5 ml of cells with 0.5 ml of 2x-IgE.

- Seed 1 ml of IgE-RBL per 16 mm well.

- Incubate plate(s) overnight at 37°C/5% CO_2.

- Next day, dilute the DNP-HSA (dinitrophenol-human serum albumin; Calbiochem) to an appropriate dilution in 1x PIPES+glucose+calcium; 0.5 ml will be needed for each well.

- Remove the plate(s) from the incubator and aspirate the media from the wells.

- Add 2 ml of 1X PIPES+glucose+calcium to each well and aspirate again.

- Repeat the last step.

- Add the 0.5 ml of diluted DNP-BSA (or 1x PIPES+glucose+calcium alone as the negative control in triplicate) to wells.

- Incubate 40 min at 37°C/5% CO_2.

- Meanwhile, label 12 x 75 tubes (round or conical bottom) to receive each supernatant and the cells themselves from the triplicate negative controls; also label 12 x 75 tubes for the beta-hexosaminidase assay.

- After the incubation, carefully transfer each supernatant from the 16 mm wells to appropriate tubes on ice.

- Add 250 µl of enzyme-free cell dissociation solution (Enzyme Free Cell Dissociation Solution Hank's Balanced Salts Based Formulation, Specialty Media Inc., Lavallette, NJ) to each of the triplicate negative control wells and incubate 5 min at 37°C.

- Transfer the 250 µl aliquots to conical bottom centrifuge tubes. Add 250 µl of PIPES+glucose+calcium to rinse each well and transfer to the appropriate tubes.

- Spin tubes 5 min at 1000 rpm and remove supernatants. Resuspend each in 500 µl of PIPES+glucose+calcium.

- Sonicate cell suspensions on ice (5 pulses); if no probe sonifier is available, freeze

thaw cells 3x using a methanol or ethanol-dry ice bath and a 37°C water bath.

- Transfer 100 µl of each supernatant or cell lysate to a 12 x 75 tube and proceed with the beta-hexosaminidase ("beta-hex") assay protocol.

Protocol for Removing RBL Cells from Flasks

- Prepare trypsin/EDTA by diluting stock 1:10 in HBSS--; filter sterilize.
- Aspirate the medium from a flask of cells.
- Add 10 ml of HBSS-- and gently rinse the flask.
- Aspirate the HBSS--.
- Add 3 ml of trypsin/EDTA and incubate 5 min at 37°C.
- Rap the flask several times to loosen the cells.
- Transfer the 3 ml to a tube.
- Rinse flask with 10 ml of the above medium and pool to the tube.

8.1.2. BETA-HEXOSAMINIDASE ASSAY

Materials

1. Beta-hex buffer

Solution 1: 0.2 M Na_2PO_4 (sodium phosphate dibasic ANHYDROUS) - 14.2 g/500 ml distilled H_2O

Note: If there is no anhydrous, use the sodium phosphate dibasic HEPTAHYDRATE ($7H_2O$), but use 26.7 grams.

Solution 2: 0.4 M citric acid monohydrate - 42.1 g/ 500 ml distilled H_2O

Mix approximately 70 ml of solution 1 with approximately 20 ml solution 2 until the pH is 4.5 (use solution 1 to raise the pH and solution 2 to lower it)

2. Beta-hex cocktail

Beta-hex buffer (from above): 90 ml

Distilled H_2O: 135 ml

p-nitrophenyl-N-acetyl-beta-D-glucosaminide (p-nitrophenyl-beta-D-2-acetamide-2-deoxy-beta-D-glucopyranoside) (Sigma N-9376): 300 mg (0.30 g); stored at -20°C

Mix until dissolved

Aliquot in 15 ml tubes, label, date, and freeze at -20°C.

3. Beta-hex STOP solution

glycine: 15.0 g/ liter

bring to pH 10.7 with 10 N NaOH (will need to add ~30 ml of NaOH)

Method

1. Place 100 µl of sample in each 12 x 75 polystyrene round-bottom tube.

2. Add 400 µl of beta-hex cocktail to each tube.

3. Cover with tin foil and incubate tubes 30 min at 37°C.

4. Add 1.5 ml of beta-hex stop to each tube.

5. Turn on the spectrophotometer and allow it to warm up 10 minutes.

6. Turn on the vacuum pump attached to the spectrophotometer.

7. Set the wavelength for 410 nm.

8. Aspirate any liquid in the spectrophotometer tubing.

9. Zero the spectrophotometer using beta-hex stop solution.

10. Aspirate the liquid from the tubing; double check reading with more beta-hex stop.

11. Aspirate the liquid from the tubing; read the absorbance at 410 nm (A410) of each sample within

one hour, remembering to aspirate the sample from the tubing between each one.

8.1.3. CALCULATION OF RESULTS

Determine the total beta-hex present in the RBL cells by adding the supernatant (S) and cell lysate (C) values for each of the triplicate negative controls and determine the mean [S+C].

Determine the % release for each supernatant by taking the beta-hex value and dividing by (S+C) x 100.

Determine the net % release by subtracting the % release value for the control from each % release value where DNP-BSA was added.

8.2. PERMEABILIZED CELL ASSAYS

The mast cell/basophil degranulation assay can also be performed using permeabilized cells, according to the protocol described below (see also Cunha-Melo et al., 1989, J. Immunol. 143:2617-2625; Ali et al., 1989, J. Immunol. 143:2626-2633; Ali et al., 1989, Biochim. Biophys. Acta 1010:88-99).

8.2.1. PROTOCOL FOR PERMEABILIZATION OF RBL-2H3 CELLS WITH STREPTOLYSIN O

(1) Buffer:

Potassium glutamate	138.7 mM
Glucose	5 mM
Potassium salt of PIPES	20 mM
Magnesium Acetate	7 mM

Make 1 litre of the above buffer, do not adjust pH, sterile filter and store at 4°C until use.

At the time of use, remove appropriate volume and add the following:

1 M EGTA to give final concentration of 1 mM.
ATP (Sigma #A2383) to a final concentration of 5 mM.
Add $CaCl_2$ (1 M) to a final concentration of 0.213 mM.
In the presence of 1 mM EGTA the free $[Ca^{2+}]$ is 100 nM.
Adjust pH to 7.0.
BSA (1 mg/ml) optional.
LiCl (10 to 20 mM) for phosphoinositide hydrolysis experiments.

(2) Streptolysin O

Streptolysin O reduced (Burrough's Wellcome; catalog #MR 16).

Add 4 ml H_2O to 40 I.U. of streptolysin O. (10 units/ml). Make aliquots and freeze immediately.

(3) Permeabilization

RBL-2H3 cells (0.2 x 10^6/ well, in growth medium) are plated in 24 well tissue culture plate and incubated overnight.

The following day, cells are washed twice with potassium glutamate (KG) buffer (500 µl).

The cells are permeabilized by exposure to streptolysin O (0.1 to 0.3 units/ml, 200-500 µl/well) for 5 to 10 min. The concentration of streptolysin O and the time required for permeabilization depends on the number of passages the cells have been cultured

for and may vary with batch of streptolysin O. A useful starting point is to permeabilize cells with 0.25 I.U./ml streptolysin for 10 min.

Streptolysin O solution is prepared just before permeabilization by dilution of the stock solution (10 I.U./ml) into prewarmed KG buffer.

After the cells are permeabilized, remove buffer by aspiration, add fresh buffer (without toxin) and perform experiment as desired to measure cell activation (e.g., measure phosphoinositide hydrolysis, or β-hexosaminidase or histamine release). Modifications of the permeabilization procedure can be made for measuring degranulation.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Weigele, Manfred
Guo, Tao
Sundaramoorthi, Raji
Dalgarno, David C.
Zydowsky, Lynne D.
Green, Jeremy
Green, Oluyinka M.

(ii) TITLE OF INVENTION: ANALOGS OF RECEPTOR TYROSINE ACTIVATION MOTIFS AND THERAPEUTIC USES THEREOF.

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pennie & Edmonds
(B) STREET: 1155 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US To be assigned
(B) FILING DATE: On even date herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Misrock, S. Leslie
(B) REGISTRATION NUMBER: 18,872
(C) REFERENCE/DOCKET NUMBER: 7337-034-999

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 790-9090
(B) TELEFAX: (212) 869-8864/9741
(C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 can only be D (Asp) or E (Glu)"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /label= note
/note= "Residue 9 can only be D (Asp) or E (Glu)"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 26
(D) OTHER INFORMATION: /label= Note
/note= "Residue 26 can only be L (Leu) or I (Ile)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Leu Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Xaa Xaa
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Tyr Glu Thr Ala Asp Gly Gly Tyr Met Thr Leu Asn Pro Arg Ala
1 5 10 15

Pro Thr Asp Asp Asp Lys Asn Thr Tyr Leu Thr Leu Pro Pro Asn Asp
20 25 30

His Val Asn Ser Asn Asn
35

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Arg Leu Lys Ile Gln Val Arg Lys Ala Asp Ile Ala Ser Arg Glu
1 5 10 15

Lys Ser Asp Ala Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr
20 25 30

Tyr Glu Thr Leu Lys His Glu Lys Pro Pro Gln
35 40

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Tyr Arg Ile Gly Gln Glu Phe Glu Arg Ser Lys Val Pro Asp Asp Arg
1 5 10 15

Leu Tyr Glu Glu Leu His Val Tyr Ser Pro Ile Tyr Ser Ala Leu Glu
20 25 30

Asp Thr Arg Glu Ala Ser Ala Pro Val Val Ser
35 40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Gln Asp Gly Val Arg Gln Ser Arg Ala Ser Asp Lys Gln Thr Leu
1 5 10 15

Leu Pro Asn Asp Gln Leu Tyr Gln Pro Leu Lys Asp Arg Glu Asp Asp
20 25 30

Gln Tyr Ser His Leu Gln Gly Asn Gln Leu Arg Arg Asn
35 40 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly His Glu Thr Gly Arg Leu Arg Gly Ala Ala Asp Thr Gln Ala Leu
1 5 10 15

Leu Arg Asn Asp Gln Val Tyr Gln Pro Leu Arg Asp Arg Asp Asp Ala
20 25 30

Gln Tyr Ser His Leu Gly Gly Asn Trp Ala Arg Asn Arg
35 40 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
1 5 10 15

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala Phe

20 25 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Asp Ala Tyr Ser Asp Ile Gly Thr Lys Gly Glu Arg Arg Arg Gly
1 5 10 15

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
20 25 30

Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala Pro Arg
35 40 45

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Lys Arg Trp Gln Asn Glu Lys Leu Gly Leu Asp Ala Gly Asp Glu
1 5 10 15

Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser
20 25 30

Met Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly Arg Tyr Gln Asp Val
35 40 45

Gly Ser Leu Asn Ile Ala Gln
50 55

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Lys Arg Trp Gln Asn Glu Lys Phe Gly Val Glu Met Pro Asp Asp
1 5 10 15

Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser
20 25 30

Met Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly Thr Tyr Gln Asp Val

35 40 45

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asp Lys Asp Asp Gly Lys Ala Gly Met Glu Glu Asp His Thr Tyr Glu
1 5 10 15

Gly Leu Asn Ile Asp Gln Thr Ala Thr Tyr Glu Asp Ile Val Thr Leu
20 25 30

Arg Thr Gly Glu Val Lys Trp Ser Val Gly Glu His Pro Gly Gln Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Lys Asp Glu Gly Lys Ala Gly Met Glu Glu Glu His Thr Tyr Glu
1 5 10 15

Gly Leu Asn Ile Asp Gln Thr Ala Thr Tyr Glu Asp Ile Val Thr Leu
20 25 30

Arg Thr Gly Glu Val Lys Trp Ser Val Gly Glu His Pro Gly Gln
35 40 45

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Lys Val Tyr Asp Lys Leu Leu Lys Arg Asn Gln Glu Thr Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Cross-links
(B) LOCATION: 5..9
(D) OTHER INFORMATION: /label= NOTE
/note= "Residue 5 and 9 are covalently bonded, which results in cyclization."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Lys Val Tyr Asp Lys Leu Leu Lys Arg Asn Gln Glu Leu Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Cross-links
(B) LOCATION: 5..9
(D) OTHER INFORMATION: /label= note
/note= "Residue 5 and 9 are covalently bonded, which results in cyclization."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Lys Val Tyr Cys Lys Leu Leu Cys Arg Asn Gln Glu Leu Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Lys Thr Tyr Lys
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asn Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Lys Thr Tyr Lys
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amine terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amide terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Lys Val Tyr Asp Lys Leu Leu Lys Arg Asn Gln Glu Thr Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amide terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group."

(ix) FEATURE:
(A) NAME/KEY: Cross-links
(B) LOCATION: 5..9
(D) OTHER INFORMATION: /label= NOTE
/note= "Residue 5 and 9 are covalently bonded, which results in cyclization."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Lys Val Tyr Asp Lys Leu Leu Lys Arg Asn Gln Glu Leu Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amide terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group"

(ix) FEATURE:
(A) NAME/KEY: Cross-links
(B) LOCATION: 5..9
(D) OTHER INFORMATION: /label= NOTE
/note= "Residue 5 and 9 are covalently bonded, which results in cyclization."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Lys Val Tyr Cys Lys Leu Leu Cys Arg Asn Gln Glu Leu Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amide terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Lys Thr Tyr Lys
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= note
/note= "Residue 1 has an amide terminal acetyl group."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /label= note
/note= "Residue 19 has a carboxy terminal amide group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asn Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Lys Thr Tyr Lys
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Asp Xaa Val Tyr Xaa Xaa Leu Xaa Xaa Arg Asn Gln Glu Xaa Tyr Glu
1 5 10 15

Thr Leu Lys

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Asp Arg Val Tyr Glu Glu Leu Asn Ile Leu Ser Ala Thr Tyr Ser
1 5 10 15

Glu Leu Glu Asp Pro Gly Glu Asn
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ser Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr Tyr
1 5 10 15

Glu Thr Leu Lys His Glu Lys
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
1 5 10 15

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
20 25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
1 5 10 15

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
20 25 30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
1 5 10 15

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
20 25 30

Arg

WHAT IS CLAIMED IS:

1. A peptide having an amino acid sequence in the range of 15-39 amino acids, and comprising the amino acid sequence (D/E)XXXXXX(D/E)XXYXXLXXXXXXXYXX(L/I) (SEQ ID NO:1), wherein X is any amino acid.

2. A peptide having an amino acid sequence in the range of 15-39 amino acids, and comprising the amino acid sequence (D/E)XXYXXLXXXXXXXYXX(L/I)X (part of SEQ ID NO:1), wherein X is any amino acid.

3. A peptide having an amino acid sequence in the range of 15-39 amino acids, and comprising an amino acid sequence selected from the group consisting of: D G G Y M T L N P R A P T D D D K N T Y L T L P (part of SEQ ID NO:2); D A V Y T G L S T R N Q E T Y E T L K (part of SEQ ID NO:3); D R L Y E E L H V Y S P I Y S A L E (part of SEQ ID NO:4); D Q L Y Q P L K D R E D D Q Y S H L Q (part of SEQ ID NO:5); D Q V Y Q P L R D R D D A Q Y S H L G (part of SEQ ID NO:6); D G L Y Q G L S T A T K D T D A L H (part of SEQ ID NO:7, 8); E N L Y E G L N L D D C S M Y E D I S (part of SEQ ID NO:9, 10); D H T Y E G L N I D Q T A T Y E D I V (part of SEQ ID NO:11); and E H T Y E G L N I D Q T A T Y E D I V (part of SEQ ID NO:12).

4. A peptide having an amino acid sequence in the range of 15-39 amino acids, and comprising an amino acid sequence selected from the group consisting of: D G V Y T G L S T R N Q E T Y E T L K (SEQ ID NO:13); Q K V Y D K L L K R N Q E T Y E T L K (SEQ ID NO:14); Q K V Y D K L L K R N Q E L Y E T L K (SEQ ID NO:15); Q K V Y C K L L C R N Q E L Y E T L K (SEQ ID

NO:16); and D G V Y T G L S T R N Q K T Y K T L K (SEQ ID NO:17); and N G V Y T G L S T R N Q K T Y K T L K (SEQ ID NO:18).

5. A peptide having 19 amino acids, and having the following amino acid sequence: D G V Y T G L S T R N Q E T Y E T L K-NH_2 (SEQ ID NO:19).

6. A peptide having 19 amino acids, and having the following amino acid sequence: Q K V Y D K L L K R N Q E T Y E T L K-NH_2 (SEQ ID NO:20).

7. A peptide having 19 amino acids, and having the following amino acid sequence: Q K V Y D K L L K R N Q E L Y E T L K-NH_2 (SEQ ID NO:21).

8. A peptide having 19 amino acids, and having the following sequence: Q K V Y C K L L C R N Q E L Y E T L K-NH_2 (SEQ ID NO:22).

9. A peptide having 19 amino acids, and having the following sequence: D G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:23).

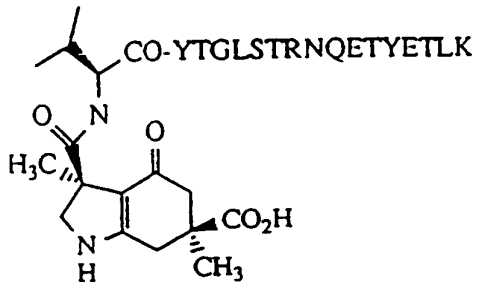
10. A peptide having 19 amino acids, and having the following sequence: N G V Y T G L S T R N Q K T Y K T L K-NH_2 (SEQ ID NO:24).

11. The peptide of claim 5, 6, or 7 which is acetylated at its amino-terminus.

12. The peptide of claim 8, 9, or 10 which is acetylated at its amino-terminus.

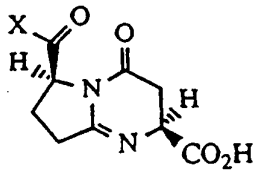
13. A peptide having an amino acid sequence in the range of 15-39 amino acids, and comprising the amino acid sequence D X V Y X X L X X R N Q E X Y E T L K (SEQ ID NO:25), wherein X is any amino acid.

14. A compound of formula Ia or the carboxy-terminal amide thereof:



(Ia)

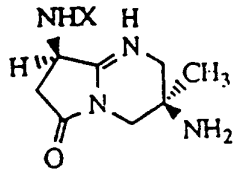
15. A compound of formula IIc



(IIc)

wherein X is OH or an amino-terminally linked peptide having a sequence in the range of 17-39 amino acids and comprising the sequence V Y T G L S T R N Q E T Y E T L K (part of SEQ ID NO:13).

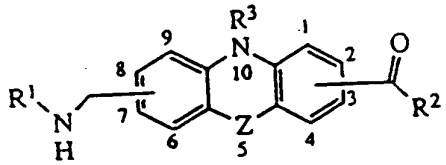
16. A compound of formula IVa:



(IVa)

wherein X is a hydrogen atom or a carboxy-terminally linked peptide having a sequence in the range of 17-39 amino acids and comprising the sequence V Y T G L S T R N Q E T Y E T L K (part of SEQ ID NO:13).

17. A compound of the general formula V:

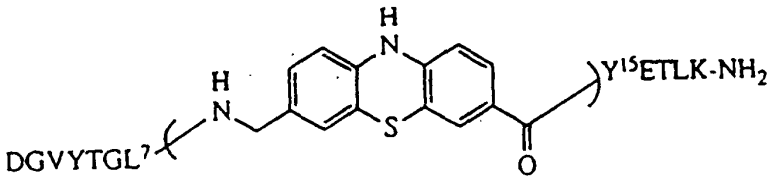


(V)

in which R^1 is an amino acid sequence of 4-25 amino acids, having a carboxy-terminal L, and a Y in the fourth position counting from the carboxy- to amino-terminal direction; R^2 is an amino acid sequence of 4-6 amino acids, having an amino-terminal Y, and an L or I in the fourth position counting from the amino- to

carboxy-terminal direction; R^3 is a hydrogen atom or an alkyl group of 1-6 carbon atoms; Z is S, SO, or SO_2.

18. The compound of claim 17 which is of formula Va:



(Va)

19. The compound of claim 17 in which R^3 is a hydrogen atom; Z is S; R^2 is Y E T L K-NH_2 (part of SEQ ID NO:17); and R^1 is D G V Y T G L (part of SEQ ID NO:17), or Q K V Y D K L (part of SEQ ID NO:19).

20. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of claim 1; and a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of claim 2, 3, or 4; and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of claim 5, 6, or 7; and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of claim 8, 9, or 10; and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of claim 13; and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 14; and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 15 or 16; and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 17; and a pharmaceutically acceptable carrier.

28. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 18 or 19; and a pharmaceutically acceptable carrier.

29. The composition of claim 20, 25, or 27 in which the pharmaceutically acceptable carrier is a propellant.

30. An inhaler containing a composition comprising the peptide of claim 1.

31. An inhaler containing a composition comprising the compound of claim 14.

32. An inhaler containing a composition comprising the compound of claim 17.

33. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 1 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

34. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 2 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

35. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 3 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

36. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 5, 6, or 7 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

37. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 8, 9, or 10 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

38. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the peptide of claim 12 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

39. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the compound of claim 14 to a subject having or suspected of having a

disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

40. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the compound of claim 15 or 16 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

41. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the compound of claim 18 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

42. A method of treating or preventing a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation comprising administering a therapeutically or prophylactically effective amount of the compound of claim 19 to a subject having or suspected of having a disease or disorder associated with undesirable or inappropriate immune system activity or inflammation.

Figure 1

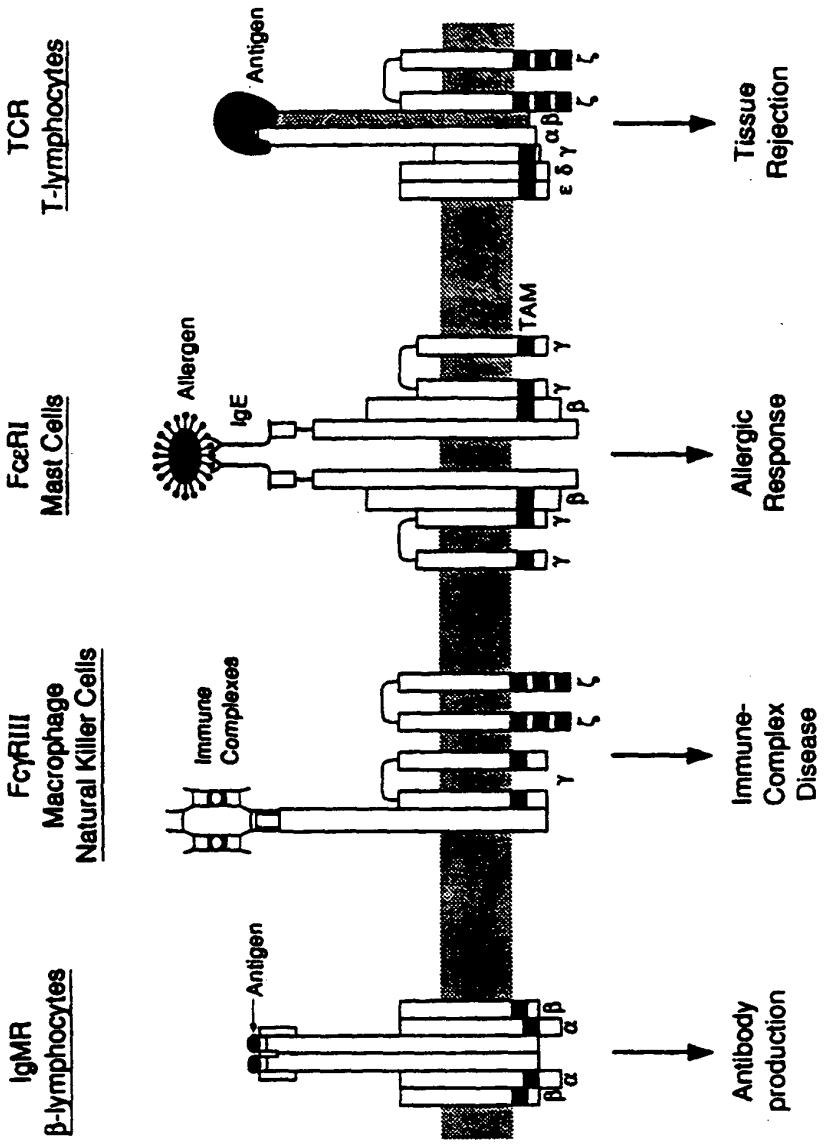

TCR
T-lymphocytes
FcεRI
Mast Cells
FcγRIII
Macrophage
Natural Killer Cells
IgMR
β-lymphocytes
Antigen
Allergen
IgE
Immune
Complexes
Antigen
TAM
Tissue
Rejection
Allergic
Response
Immune-
Complex
Disease
Antibody
production

Figure 2

FcεRI

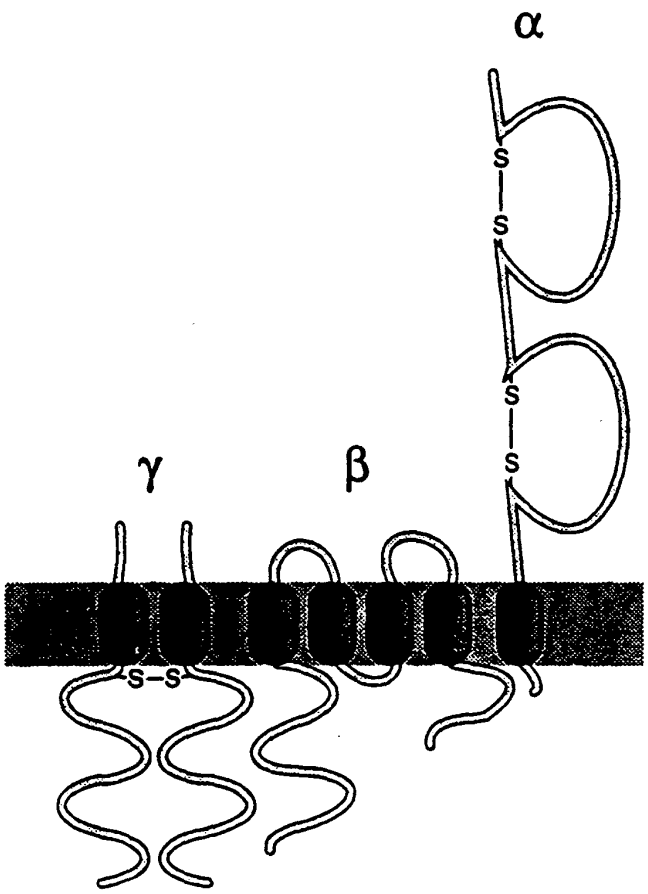


FIGURE 3

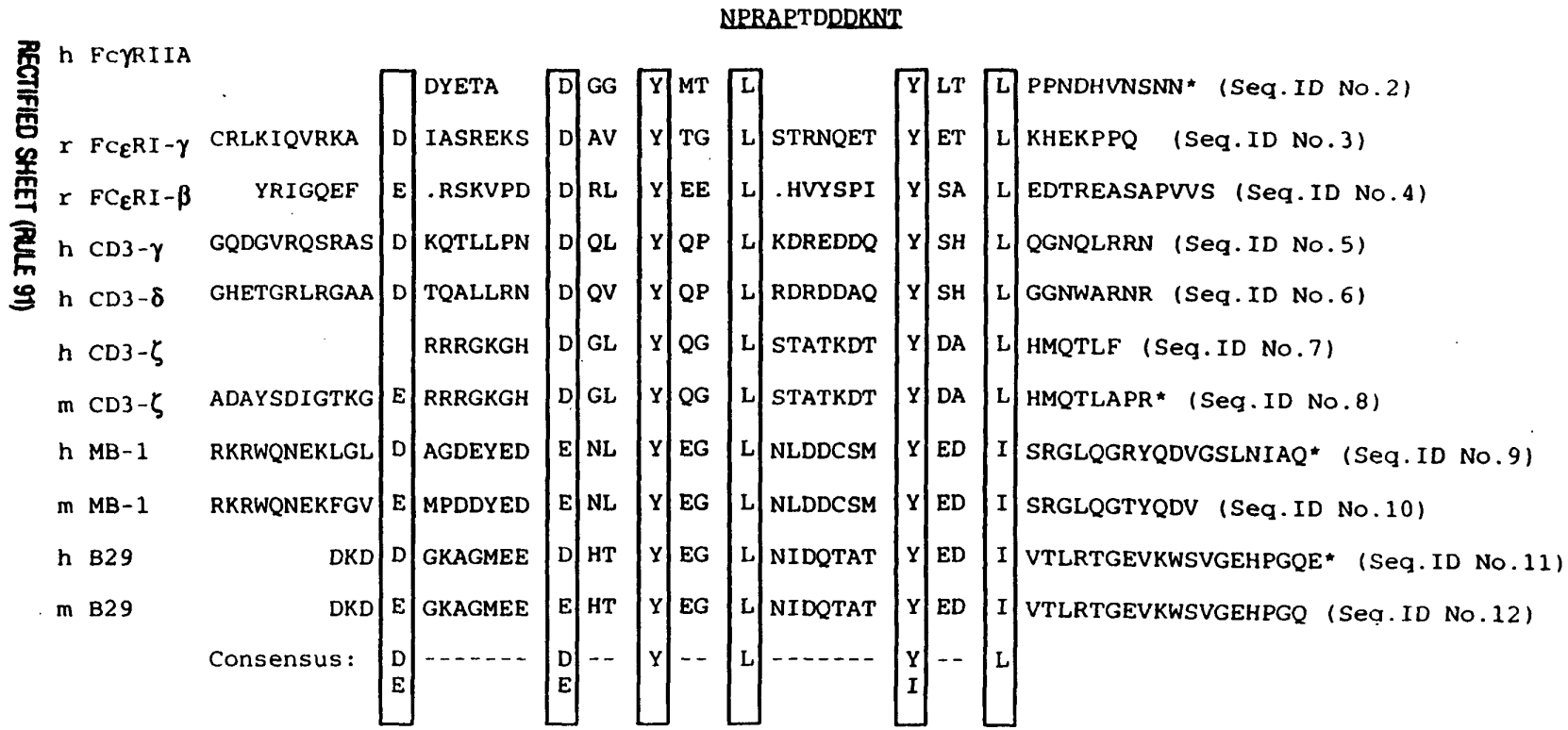


FIGURE 4

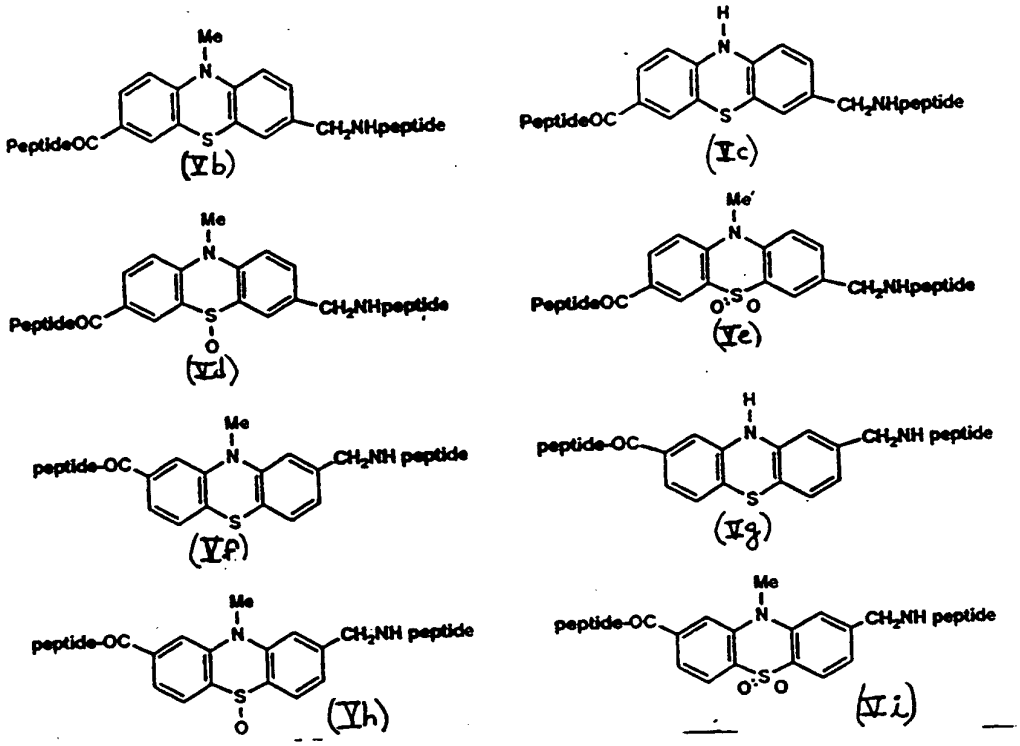
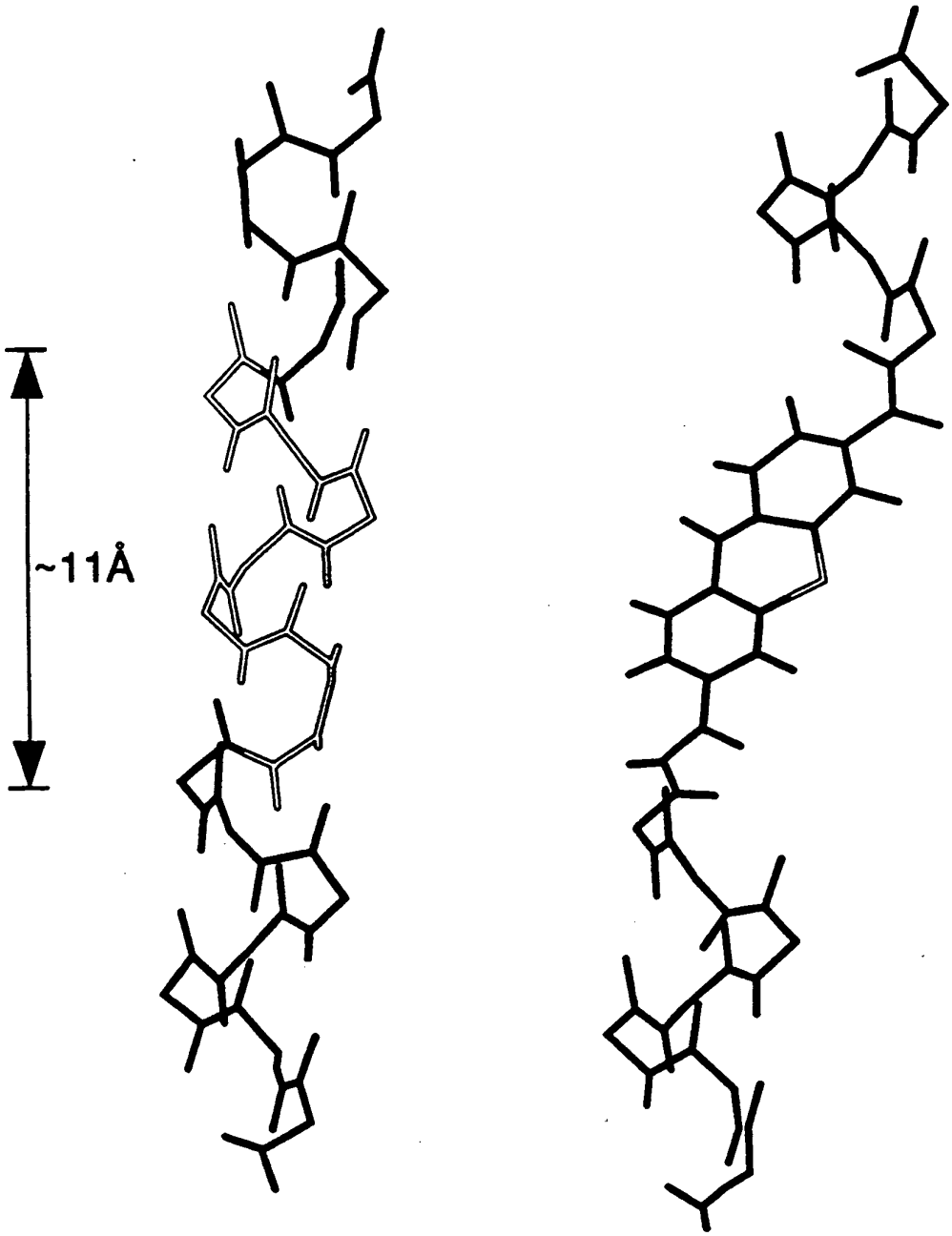
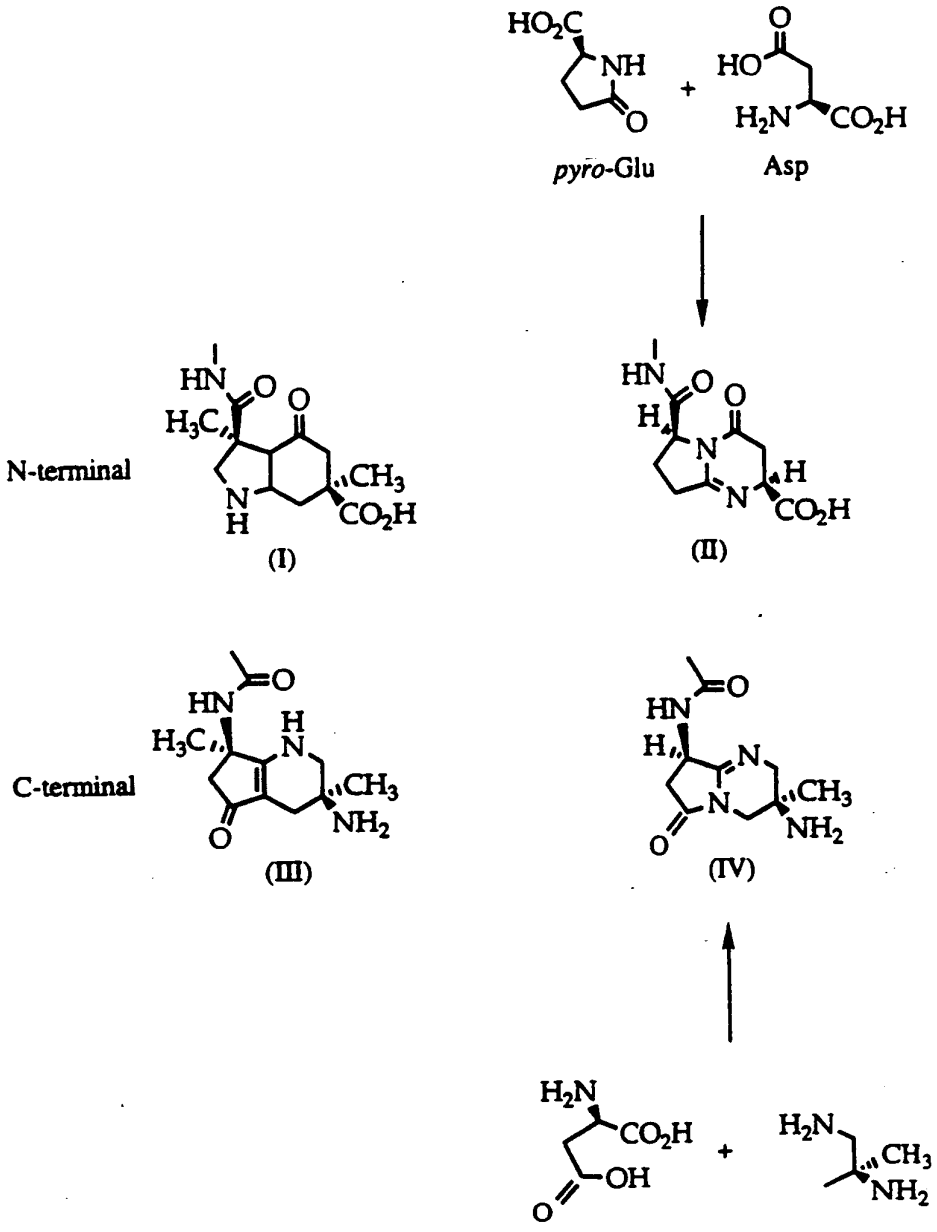

Me
N
S
PeptideOC
CH2NHpeptide
(Ib)
H
N
S
PeptideOC
CH2NHpeptide
(Ic)
Me
N
S
O
PeptideOC
CH2NHpeptide
(Id)
Me
N
S
O O
PeptideOC
CH2NHpeptide
(Ie)
Me
N
S
peptide-OC
CH2NH peptide
(If)
H
N
S
peptide-OC
CH2NH peptide
(Ig)
Me
N
S
O
peptide-OC
CH2NH peptide
(Ih)
Me
N
S
O O
peptide-OC
CH2NH peptide
(Ii)

Figure 5



Hybrid Helices

FIGURE 6



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01025

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 327, 328, 329, 345; 514/12, 13, 14, 258; 424/88; 546/253; 530/868

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS; FILE REGISTRY; FILECA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANNUAL REPORTS IN MEDICINAL CHEMISTRY, VOLUME 24, ISSUED 1989, B.A. MORGAN ET AL. "APPROACHES TO THE DISCOVERY OF NON-PEPTIDE LIGANDS FOR PEPTIDE RECEPTORS AND PEPTIDASED", PAGES 243-252, ESPECIALLY PAGE 249, COMPOUND 22.	17
X	PROC. NATL. ACAD. SCI. USA, VOLUME 84, NUMBER 24, ISSUED DECEMBER 1987, H. SATO ET AL. "CLOSE LINKAGE OF THE MOUSE AND HUMAN CD3γ- AND δ-CHAIN GENES SUGGESTS THAT THEIR TRANSCRIPTION IS CONTROLLED BY COMMON REGULATORY ELEMENTS", PAGES 9131-9134, ESPECIALLY PAGE 9133, FIGURE 22.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 MAY 1994	Date of mailing of the international search report MAY 13 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KAY KIM, PH.D. Jill Warden for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US94/01025

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07K 5/02, 5/10, 7/02, 7/08, 7/10; C07D 39.70; A61K 31/495, 37/02, 37/64, 39/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/324, 325, 326, 327, 328, 329, 345; 514/12, 13, 14, 258; 424/88; 546/253